FORMULATION, CHARACTERIZATION AND EVALUATION OF NYSTATIN NANOSPONGE GEL FOR THE TREATMENT OF CANDIDIASIS

A Dissertation submittedto

THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY
CHENNAI–600032

In partial fulfillment of the requirements for the award of degree of

MASTEROF PHARMACY

Submitted by

Register no:261411263

under the guidance of

Prof.K.Elango,M.Pharm.,(Ph.D.),

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COLLEGEOFPHARMACY

MADRAS MEDICAL COLLEGE

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APRIL-2016

DEPARTMENT OF PHARMACEUTICS
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TAMILNADU

CERTIFICATE

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Place: Chennai -03.

Date:

(Dr.A.Jerad Suresh, M. Pharm.,M.B.A.,)

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Place: Chennai– 03

Date:

(K.Elango)

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ABBREVIATIONS

DDS	Drug Delivery System
NDDS	Novel Drug Delivery System
mg	Milligram
ng	Nanogram
ml	Millilitre
nm	Nanometer
L	Litre
PLA	Poly Lactic Acid
PLG	Poly D,L- Glycolide
PLGA	Poly D,L- Lactide co glycolide
PCA	Poly Cyano Acrylate
SAS	Super Critical Anti-solvent
RESS	Rapid Expansion of Critical Solution
DOPE	Di Oleolyl Phosphatidyl Ethanolamine
GNP	Gliadin Nano Particles
CGNP	Clarithromycin Gliadin Nano Particles
OGMP	Omeprazole Gliadin Nano Particles
BBB	Blood Brain Barrier
CNS	Central Nervous System
LDL	Low-density Lipoprotein
IBCA	Isobutyl 1-cyano acrylate
PVA	Polyvinyl Alcohol
UV	Ultra-violet
HPLC	High Pressure Liquid Chromatography
SEM	Scanning Electron Microscope

TEM	Transmission Electron Microscope
FT-IR	Fourier Transform Infra Red spectroscopy
XRD	X-ray diffraction
DSC	Differential Scanning Calorimetry
BSA	Bovine serum albumin
OPC	Oropharyngeal Candidiasis
VVC	Vulvovaginal Candidiasis
HIV	Human Immuno virus
OTC	Over the counter
FDA	Food and Drug Administration
PMMA	Polymethyl methacrylate
EC	Ethyl cellulose
PCS	Photon Correlation Spectroscopy
RH	Relative Humidity
ICH	International Conference on Hormonisation
^{o}C	Degree Celsius

TABLE OF CONTENT

1. *INTRODUCTION*

1. DRUG DELIVERY SYSTEM

The Drug delivery system refers to a system for transporting pharmaceutical compound in the body to provide a desired and required therapeutic effect. Drug delivery is a concept integrated with dosage form and route of administration of pharmaceutical products. The technologies involved in the formulation of pharmaceutical products modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Drug release is from: diffusion, degradation, swelling, and affinity-based mechanisms. Most common routes of administration are non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes.[1]

Newer development of pharmaceutical compounds in the form of targeted delivery system, in which the drug is only active in the target area of the body (for example, in cancerous tissues, in brain, in colon), sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation.[1]

2. NOVEL DRUG DELIVERY SYSTEM

The aim of Novel Drug Delivery System is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug-delivery system should deliver drug at a rate control by the necessarily of the body over a specified term of treatment.[2]

This idealized objectives witch to the two aspects most important to drug delivery areas follows,[2]

I. **Spatial Drug Delivery:**

Targeting a drug to a particular organ or tissue.

II. **Temporal Drug Delivery:**

The drug delivery rate to the target tissue is controlled.

The prime area so research and development for NDDS are:

Liposomes

Niosomes

Nanoparticles

Transdermaldrugdelivery

Implants

Oralsystem

Microencapsulation/Microcapsules

Polymer in drug delivery

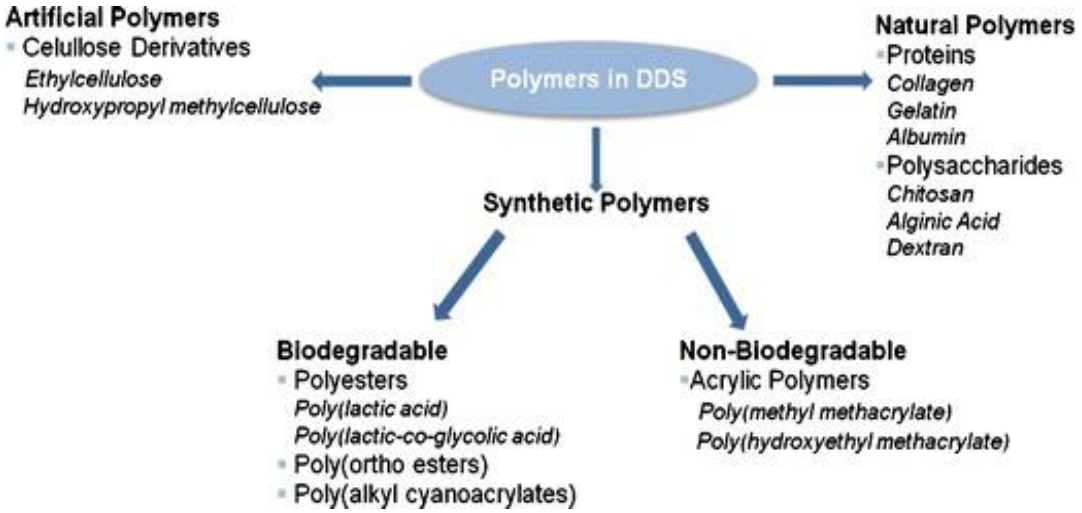


Figure-1 :Polymers used in Drug Delivery system.

Efficacy of a drug can be altered by the method of drug delivery into the body. Some drugs has an optimum concentration in the body and produce maximum therapeutic level, concentration above or below can produce toxic or no therapeutic action. Slow efficacy of drug in severe diseases has increases the need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. To solve this problem newer development of pharmaceutical compounds were generated to controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, bio recognition, and efficacy of drugs. The drug delivery systems (DDS), based on interdisciplinary approaches that combine polymer science,

pharmaceutics, bioconjugate chemistry, and molecular biology are called as Novel drug delivery system (NDDS).[3]

To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Drug carriers can named as soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g., pH- or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest).[3]

ADVANTAGES OF NOVEL DRUG DELIVERY SYSTEM[4]

- Minimization of drug degradation and loss.
- Reduction of dosing frequency.
- Extension of the duration of action and bioavailability of the drug.
- Prevention of drug's adverse side-effects.
- Minimization of drug concentration fluctuations in plasma level.
- Improved drug utilization.
- Improved patient compliance.

DISADVANTAGES OF NOVEL DRUG DELIVERY SYSTEM[4]

- High cost of final product.
- Patients discomfort with DDS device usage.
- Possibility of toxicity of the materials.
- Harmful degradation products.
- Necessity of surgical intervention either on systems application or removal.

CLASSIFICATION OF DRUG DELIVERY SYSTEM:[5]

Classification of NDDS based on Physical means

1) Osmotic Pressure Activated
2) Hydrodynamic pressure activated
3) Vapor pressure activated
4) Mechanically activated
5) Magnetically activated
6) Sonophoresis
7) Iontophoresis
8) Hydration activated

Classification of NDDS based on Chemical means

1) Hydrolysis activated
2) Ion activated
3) pH activated

Targeting the drug to a specific site by two mechanisms that is (i) Active Targeting and (ii) passive Targeting.[6]

Potential release mechanism[6] involves (i) Desorption of surface -bound or adsorbed drugs, (ii) diffusion through the carrier matrix, (iii) diffusion through the carrier wall(in case of nanocapsules),(iv) carrier matrix erosion, and (v) a combined erosion /diffusion process.

Sustained (or) continuous release[6] of a drug involves polymers that release the drug at a controlled rate due to diffusion out of the polymer or by degradation of the polymer over time.

Pulsatile release[6] is often the preferred method of drug delivery, as it closely mimics the way by which the body naturally produce hormones such as insulin.

Colloidal drug carrier system[6] such as micellar solution, vesicle and liquid crystal dispersion as well as nanoparticle consisting of small particles of 10-400 nm diameter shows great promise as drug delivery system.

Micelles[6] formed by self - assembly of amphiphilic block co-polymer (5-50 nm) in aqueous solutions are of great interest for drug delivery application. They can be physically entrapped in the core of block copolymer micelles and transported at concentration that can exceed their intrinsic water solubility.

Liposomes[3] are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in**niosomes**. The drugs that are encapsulated in a nanocage-functionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes.

Dendrimers[3] are nanometer-sized, highly branched and monodisperse macromolecules with symmetrical architecture. They consist of a central core, branching units and terminal functional groups. The core together with the internal units, determine the environment of the nanocavities and consequently their solubilizing properties, whereas the external groups the solubility and chemical behaviour of these polymers.

Liquid Crystals[3] combine the properties of both liquid and solid states. They can be made to form different geometries, with alternative polar and non-polar layers (i.e., a lamellar phase) where aqueous drug solutions can be included.

Nanoparticles[7] can be defined as particulate dispersion or solid particle with a size range in 10-1000 nm.The drug is dissolved , entrapped, encapsulate or attached to a nanoparticle matrix. Depending upon the method of preparations, nanoparticle, nanospheres, or nanocapsules can be obtained.

The **primary goals** for research of nano-bio-technologies in drug delivery include:[8]

- More specific drug targeting and delivery,
- Reduction in toxicity while maintaining therapeutic effects,
- Greater safety and biocompatibility, and
- Faster development of new safe medicines.

Method of preparation:

1. Dispersion of preformed polymers.[9]
    a) Solvent evaporation method.[11]
    b) Spontaneous emulsification or solvent diffusion method.[11]
2. Polymerization of monomers[9] and
3. Ionic gelation or coacervation of hydrophilic polymers.[9]
4. Supercriticalfluid technology.[11]

1.Dispersion of preformed polymers:[11]

It is the most common method used to prepare nanoparticle from poly(lactic acid) (PLA), poly (D L Glycolide), PLG, Poly(D,L-lactide-co-glycolide) PLGA, and Poly(cyanoacrylates) PCA.

a) Solvent evaporation method:[11]

Organic solvent such as Dichloromethane, Chloroform, or ethyl acetate are used to dissolve the polymer which is also used as solvent to dissolve hydrophobicdrugs. The drug dissolved or dispersed in a polymer solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water emulsion. Once a stable emulsion is formed the solvent is subjected to evaporation either by reducing the pressure or by continuous stirring. High speed homogenizer or Ultra sonicator is used for the preparation of small uniform sized particle.

b) Spontaneous emulsification or solvent diffusion method:[11]

This is a modification of solvent evaporation method. In this the water miscible solvent along with water immiscible organic solvent is used as an oil phase. An interfacial turbulence is formed between the two phases due to spontaneous diffusion of immiscible solvents leading to formation of small particles. By increasing the concentration of water miscible solvent decrease in the particle size can be achieved. Both solvent evaporation and solvent diffusion methods can be used for the hydrophobic and hydrophilic drugs.

2.**Polymerization of monomers:[11]**

In this method monomers are polymerized to form nanoparticle in an aqueous solution in which drug maybe dissolved. Drugs may also be incorporated by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified by ultracentrifugation and resuspending in an isotonic surfactant - free medium. This technique is used for preparing polybutylcyanoacrylateor poly (alkylcyanoacrylate) nanoparticle.

3.**Ionic gelation or coacervation of hydrophilic polymers :**

The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO or PPO) and the other is the polyanion sodium tripolyphospate. Positively charged amino group in chitosan interacts with negatively charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two phases , whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction condition at room temperature.

4.Supercritical fluid technology:

Supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro and nanoparticles. Supercritical fluids are environmentally safe.

A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO2(SCCO2)is the most widely used supercritical fluid because of its mild critical conditions (Tc=31.1°C,Pc=73.8bars),non toxicity, non-flammability, and lowprice. The most

common processing techniques involving supercritical fluids are supercritical anti- solvent(SAS) and rapid expansion of critical solution (RESS).

Nanoparticle [10]can be formulated, as injections consisting of spherical amorphous particles which do not aggregate, hence they can be safely administered by the intravenous route. Since no cosolvent is used to solubilize the drug, the overall toxicity of the formulation is decreased.

Nanoparticles represent very promising carrier system for the targeting of anti-cancer agents to tumors. Nanoparticles exhibit a significant tendency to accumulate in a number of tumors after iv injection. Nanoparticles can also be used in Brain Drug Targeting. Poly (butyl cyanoacrylate) nanoparticles represent the only nanoparticles that were so far successfully used for *in vivo* delivery of drugs to brain. This polymer has the advantage that it is very rapidly biodegradable. The first drug that was delivered to brain using nanoparticles was the Hexapeptide Dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), a Leu-enkephalin analouge with opioid activity. Other drugs that have successfully been transported into the brain are loperamide, tubocurarine, and doxorubicin. Nanoparticles mediated drug transport to the brain depends on the over coating of the particles with polysorbates, especially polysorbate 80.

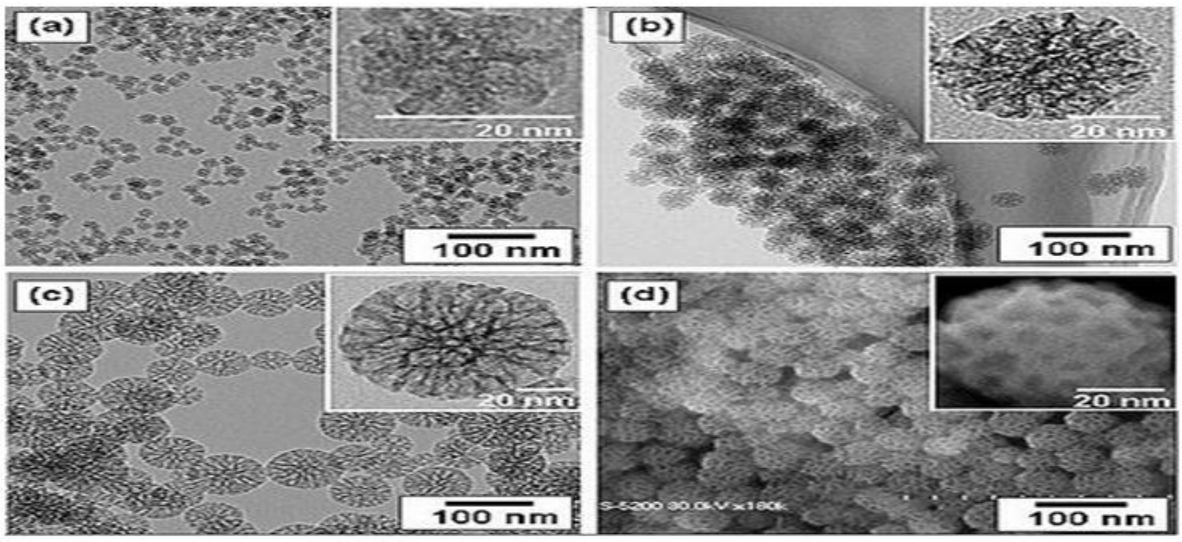


Figure-2 :(a, b, and c) images of prepared mesoporous silica nanoparticles with mean outer diameter: (a) 20nm, (b) 45nm, and (c) 80nm. (d) image corresponding to (b). The insets are a hig magnification of mesoporous silica particle.

TYPES OF NANOPARTICLES

1. **Quantum Dot**

   A**quantum dot** is a semiconductor nanostructure that confines the motion of conduction band electrons, valence band holes, or excitons (pairs of conduction band electrons and valence band holes) in all three spatial directions. The confinement can be due to electrostatic potentials (generated by external electrodes, doping, strain, impurities), due to the presence of an interface between different semiconductor materials (e.g. in the case of self-assembled quantum dots), due to the presence of the semiconductor surface (e.g. in the case of a semiconductor nanocrystal), or to a combination of these. A quantum dot has a discrete quantized energy spectrum. A quantum dot contains a small integer number (of the order of 1-100) of conduction band electrons, valence band holes, or excitons, i.e., an integer number of elementary electric charges.

   Quantum dots can be contrasted to other semiconductor nanostructures:
   1) Quantum wires, which confine the motion of electrons or holes in two spatial directions and allow free propagation in the third.
   2) Quantum wells, which confine the motion of electrons or holes in one direction and allow free propagation in two directions.

2. **Nanocrystalline silicon**

   Nanocrystallinesilicon(**nc-Si**) - an allotropic form of silicon - is similar to amorphous silicon (a-Si), in that it has an amorphous phase. Where they differ, however, is that nc-Si has small grains of crystalline silicon within the amorphous phase. This is in contrast to polycrystalline silicon (poly-Si) which consists solely of crystalline silicon grains, separated by grain boundaries. nc-Si is sometimes also known as **microcrystalline silicon** (μc-Si) The difference comes solely from the grain size of the crystalline grains. Most materials with grains in the micrometre range are actually fine- grained polysilicon, so nanocrystalline silicon is a better term.

3. **Photonic crystal**

   Photonic crystals are periodic dielectric or metallo-dielectric (nano) structures that are

designed to affect the propagation of electromagnetic waves (EM) in the same way as the periodic potential in a semiconductor crystal affects the electron motion by defining allowed and forbidden electronic energy bands.

Since the basic physical phenomenon is based on diffraction, the periodicity of the photonic crystal structure has to be in the same length-scale as half the wavelength of the EM waves i.e. ~300 nm for photonic crystals operating in the visible part of the spectrum. This makes the synthesis cumbersome and complex. To circumvent nanotechnological methods with their big and complex machinery, different approaches have been followed to grow photonic crystals as self-assembled structures from colloidal crystals.

Photonic crystals are attractive optical materials for controlling and manipulating the flow of light. They are of great interest for both fundamental and applied research, and are expected to find commercial applications soon.

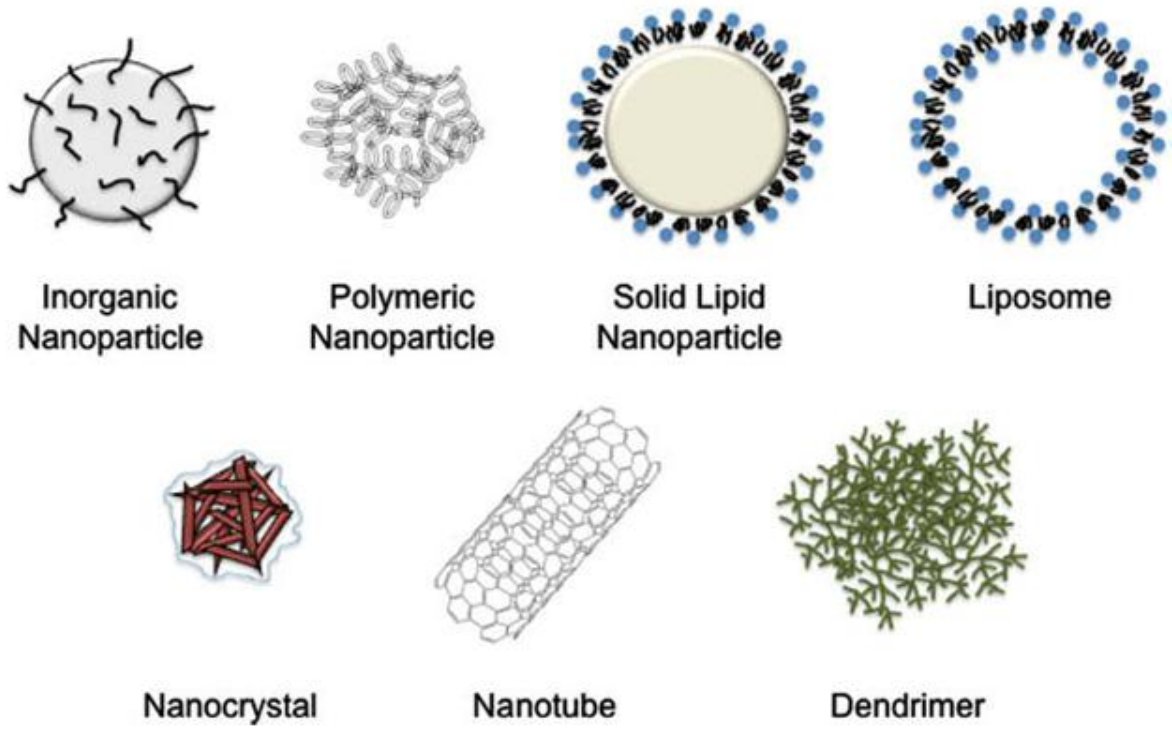


Figure-3: STRUCTURE OF NANOPARTICLES

4. Liposomes

A **liposome** is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drugs or genetic material into a cell. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg, phosphatidyl ethanolamine), or of pure components like DOPE (di oleolyl phosphatidyl ethanolamine).

The lipid bilayer can fuse with other bilayers (e.g., the cell membrane), thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs,(which would normally be unable to diffuse through the membrane), they can be (indiscriminately) delivered past the lipid bilayer.

5. Gliadin nanoparticles

In an effort to improve bioavailability anti-H.pylori effects of antibiotics, mucoadhesive gliadin nanoparticles (GNP) which have the ability to deliver the antibiotics at the sites of infection were prepared. GNP bearing clarithromycin(CGNP) and omeprazole(OGNP) were prepared by desolvation method.

in vivo gastric mucoadhesive studies confirmed the strong mucoadhesive propensity and specificity and specificity of gliadin nanoparticles towards stomach. Gliadin nanoparticles show a higher tropism for the gastrointestinal regions and their presence in other intestinal regions is very low. This high capacity to interact with the mucosa may be explained by gliadin composition.

In fact, this protein is rich in neutral and lipophilic residues. Neutral amino acid can promote hydrogen bonding interaction with the mucosa whereas the lipophilic components can interact within biological tissue by hydrophilic interaction. The related protein gliadin possessing an amino and disulphide groups on the side chain has a good probability of developing bonds with mucin gel.

6. Polymeric Nanoparticles

Polymeric nanoparticles have been invented by Speiser et al. They represent interesting alternative as drug delivery systems to liposomes. They usually exhibit a long shelf life and a good stability on storage.

These are superior to liposomes in targeting them to specific organs or tissues by adsorbing and coating their surface with different substances.

Nanoparticles can be prepared either from preformed polymers, such as polyesters (i.e. polylactic acid), or from a monomer during its polymerization, as in the case of alkyl-cyanoacrylates.

Most of the methods based on the polymerization of monomers consists in adding a monomer into the dispersed phase of an emulsion, an inverse microemulsion, or dissolved in a non-solvent of the polymer.

7. Solid Lipid Nanoparticles (SLN)

Solid lipid nanoparticles have been developed as alternative delivery system to conventional polymeric nanoparticles. SLNs are sub-micron colloidal carriers (50-1000nm) which are composed of physiological lipid, dispersed in water or in an aqueous surfactant solution.

SLNs combine advantages of polymeric nanoparticles, fat emulsions and liposomes, but avoid some of their disadvantages. They are biodegradable, biocompatible and non-toxic.

8. **Nanospheres**[16]

Nanospheres may be defined as solid core spherical particulates, which are nano in size. They contain drug embedded within the matrix or adsorbed on to the surface.

9. Nanocapsules[16]

Nanocapsules are the vesicular system in which drug is essentially encapsulated within central volume surrounded by an embryonic continuous polymeric sheath.

10. Others[10]**:**

Gold nanoparticles stabilized by thiol functionality are extraordinarily stable and therefore are a great system for studying nanostructure formation. They have many applications. Because gold nanoparticles are so easy to synthesize they have been studied intensely in recent years.

A common synthesis involves the reduction of a gold salt in the presence of capping agent molecules such as thiols, citrates or phosphines. The functionalities of these capping agents can be altered to yield various chemical properties.

Advantages of Nanoparticles

Some of the advantages of using nanoparticles as a drug delivery system are as follows;

1. Ease of manipulation of the particle size and surface characteristics of nanoparticles so as to achieve both passive and active drug targeting after parenteral administration.
2. The nanoparticle surface can be modified to alter bio distribution of drugs with subsequent clearance of the drug so as to achieve maximum therapeutic efficacy with minimal side effects of the drug.
3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents.
4. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
5. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
6. Liposomes and polymer based nanoparticulates are generally biodegradable, do not accumulate in the body and so are possibly risk free.
7. Small sized nanoparticles can penetrate through smaller capillaries, which could allow efficient drug accumulation at the target sites.
8. Various routes of administration are including oral, nasal, parenteral, intra-ocular etc.

Disadvantages

In spite of these advantages nanoparticles do have limitations like,

1. Altered physical properties which lead to particle–particle aggregation, making physical

handling of nanoparticles difficult in liquid and dry forms due to smaller size and larger surface area.

2. Smaller the particles size greater the surface area and this property makes nanoparticles very reactive in the cellular environment.
3. Small particles size results in limited drug loading and burst release. These practical problems have to be sorted out before nanoparticles can be used clinically or made commercially available.

ApplicationsofNanoparticles[12]

In Anti-Microbial Techniques :

One of the earliest nanomedicine applications was the use of nanocrystalline silver which is as an antimicrobial agent for the treatment of wounds, a nanoparticle cream has been shown to fight infections.

In Tumor targeting using :

The rational of using nanoparticles for tumor targeting is based on (1) nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active nanoparticles. (2) Nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ.

In Gene delivery :

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system.

In Cell Repair :

Nanorobots could actually be programmed to repair specific diseased cells, functioning in a similar way to antibodies in our natural healing processes.

In brain targeting :

The blood-brain barrier(BBB)is the most important factor limiting the development of new drugs for the central nervous system. Relatively impermeable endothelial cells characterize the BBB with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and canal so reduce the brain concentration of lipid- soluble molecules by the function of enzymes or efflux pumps. Consequently, the BBB only permits selective transport of molecules that are essential for brain function. Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle interaction with specific receptor- mediated transport systems in the BBB.[7]

Nanoparticles can get access to the brain by two different mechanisms, ie, (1) trans synaptic transport after inhalation through the olfactory epithelium, and (2) uptake through the blood-brain barrier.[8]

In some cases it is reported to mimic molecules that would normally be transported to brain. For example, polysorbate-coated nanoparticles are thought to mimic low-density lipoprotein (LDL), allowing them to be transported across the capillary wall and into the brain by hitching a ride on the LDL receptor.[13]

In Topical drug delivery:

Nanoemulgel[15]:

When nanoemulsion is incorporated into gel it is called as nanoemul gel. Nanoemulsions are thermodynamically stable transparent (translucent)dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size of less than100nm. Nanoemulsion formulations possess improved transdermal and dermal delivery properties *in-vitro* as well as *in-vivo*. Nanoemulsions have improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions and gels e.g.Carvedilol nanoemul gel was prepared using oleicacid and isopropyl myristate (3:1) as oil phase. Tween20

and Carbitol were used as surfactant and cosurfactant respectively. Carbopol934 was used as gelling agent[15].

Nanosponge:[17,18]

Nanosponges are tiny mesh – like nanoporous particular structure in which a large variety of substances can be encapsulated or suspended, and then be incorporated into a dosage form. They have a proven spherical colloidal nature, reported to have a very high solubilization capacity for poorly soluble drugs by their inclusion and non-inclusion behavior. Nanosponges have recently been developed and proposed for drug delivery. Nanosponges can solubilize poorly water soluble drug and provide prolonged release as well as improving drugs bioavailability. Nanosponges are able to load both hydrophilic and hydrophobic drug molecules because of their inner hydrophobic cavities and external hydrophilic branching, thereby offering unparalleled flexibility .Nanosponges are more like a three- dimensional network or scaffold. The backbone is a long length of polyester which is mixed in solution with small molecules called crosslinkers that act like tiny grappling hooks to fasten different parts of the polymer together.

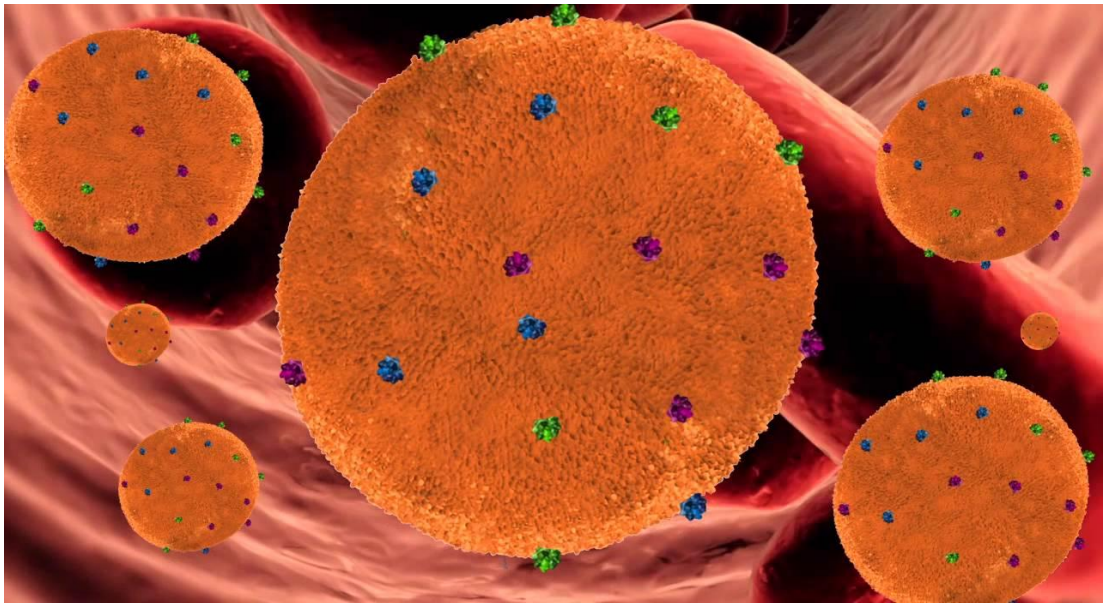


Figure-4 : STRUCTURE OF NANOSPONGE

Advantages of Nanosponge:[18,42]

1.Targeted site specific drug delivery.

2. Can be used to mask unpleasant flavours and to convert liquid substances to solids.

3. Less harmful side effects (since smaller quantities of the drug have contact with healthy tissue).

4.Nanosponge particles are soluble in water, so the hydrophobic drugs can been capsulated with in the nanosponge, after mixing with a chemical called an adjuvant reagent.

5. Particles can be made smaller or larger by varying the proportion of cross-linkerto the polymer.

6. Production through fairly simple chemistry called "click chemistry"(methods for making the Nanosponge particles and for attaching the linkers).

7. Easy scale-up for commercial production.

8.The drug profiles can be tailored from fast, medium to slow release, preventing over or under-dosing of the therapy.

9.The material used in this system can provide a protective barrier that shields the drug from premature destruction with in the body.

10.Improved stability, increased elegance and enhanced formulation flexibility.

11.Nanosponges systems are non-irritating , non-mutagenic, non-allergenic and non-toxic.

12.These are self sterilizing as the average pore sizeis 0.25μm, where bacteria cannot penetrate.

13.Hydrophobic drugs can be encapsulated within the Nanosponge.

14.Extended release-continuous action up to 12 h.

15.Biodegradable.

Disadvantages

1. The main disadvantage of these nanosponges is their ability to include only small molecules.

2. The nanosponges could be either Para crystalline or in crystalline form. The loading capacity of nanosponges depends mainly on degree of crystallization. Para crystalline nanosponges can show different loading capacities.
3. The nanosponges can be synthesized to be of specific size and to release drugs overtime by varying the proportion of cross linker to polymer.

Characteristic features of nanosponges[56].

- Nanosponges exhibit a range of dimensions (1 μm or less) with tunable polarity of the cavities. Nanosponges of specific size and adjustable polarity can be synthesized by varying the crosslinker to polymer proportion.
- They could be either para-crystalline or in crystalline form, depending on the process conditions. Crystal structure of nanosponges plays a very important role in their complexation with drugs.
- The drug loading capacity of nanosponges mainly depends on the degree of crystallization. Para-crystalline nanosponges have shown various drug loading capacities.
- They are nontoxic, porous particles insoluble in most organic solvents and stable at high temperatures up to 300 °C.
- Nanosponges as formulations are stable over the pH range of 1 to 11 and temperature up to 130 °C.
- They form clear and opalescent suspensions in water and can be regenerated by simple thermal desorption, extraction with solvents, by the use of microwaves and ultrasounds.
- Their 3D structure enables capture, transportation and selective release of a vast variety of substances. They can be targeted to different sites due to their ability to be linked with different functional groups. Chemical linkers enable nanosponges to bind preferentially to the target site. They form inclusion and non-inclusion complexes with different drugs.
- Magnetic properties can be also imparted to nanosponges (by adding magnetic particles into the reaction mixture).

Mechanism of Drug Release[18]

The sponge particles have an open structure and the active is free to move in and out from the particles and into the vehicle until equilibrium is reached . In case of topical delivery, once the finished product is applied to the skin, the active that is already in the vehicle will be

absorbed into the skin, depleting the vehicle, which will become unsaturated, therefore disturbing the equilibrium. This will start a flow of the active from the sponge particle into the vehicle and from it to the skin until the vehicle is either dried or absorbed .Even after that the sponge particles retained on the surface of stratum corneum will continue to gradually release the active to the skin, providing prolonged release overtime.

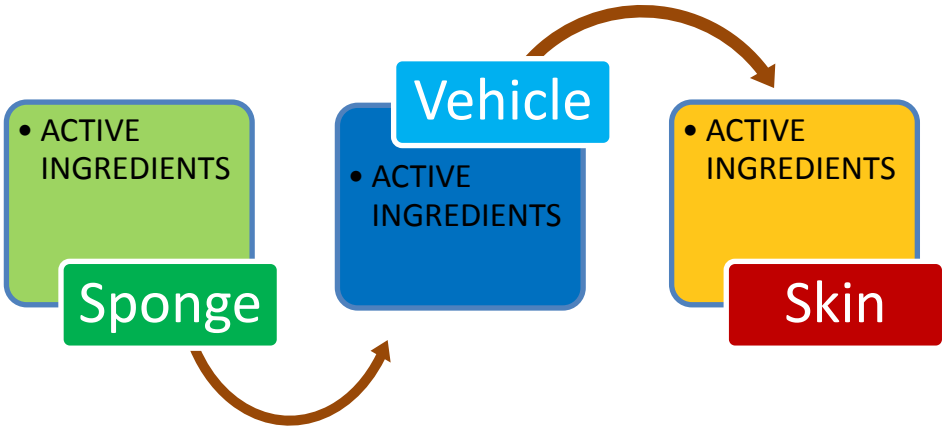


Figure -5: drug release mechanism in Topical drug delivery.

FACTORS AFFECTING DRUG RELEASE FROM NANOSPONGES[18]:

- Physical and chemical properties of entrapped actives.
- Physical properties of sponge system like pore diameter, pore volume, resiliency etc.
- Properties of vehicle in which the sponges are finally dispersed.
- Particle size, pore characteristics, composition can be considered as imperative parameters.
- External triggers like temperature, pressure, and solubility of actives.

  Pressure: Pressure or rubbing can release active ingredient from Nanosponges onto skin.

  Temperature: Some entrapped actives can be too viscous at room temperature to flow spontaneously from sponges onto the skin but increased skin or environment can result in increased flow rate and ultimately drug release.

  Solubility: Sponges loaded with water soluble drug like antiperspirants and antiseptic release the ingredients in the presence of water.

The nanosponges are encapsulate the drug molecule within its core. By the method of associating with drugs, the nanoparticles can be classified into[74]:

1. Encapsulating nanoparticles:- These are represented by nanosponges and nanocapsules. Nanosponges such as alginate nanosponges, which are sponge like structure that carry the drug molecules. Nanocapsules such as poly(isobutyl-cyanoacrylate) (IBCA) are also encapsulating nanoparticles. They can entrap drug molecule in their aqueous core.
2. Complexing nanoparticles:- These nanoparticle attract the molecule by electrostatic charges.
3. Conjugating nanoparticles:- These nanoparticles links to drug through strong covalent bonds.

PREPARATIONOFNANOSPONGES[21].

Nanosponges are prepared depending on type of delivery system, polymers and nature of drug and solvents. Various approaches used for formation of Nanosponges are:

1.Polymerization:

The polymerization process leads to the formation of a reservoir type of system, which opens at the surface through pores. A solution of non-polar drug is made in the monomer, to which aqueous phase, usually containing surfactant and dispersant to promote suspension is added. Polymerization is effected, once suspension with the discrete droplets of the desired size is established; by activating the monomers either by catalysis or increased temperature.

2.Quasi-emulsionsolventdiffusion

The Nanosponges can also be prepared by quasi-emulsion solvent diffusion method using the different polymer amounts. To prepare the inner phase, EudragitRS100 was dissolved in suitable solvent. Then, drug can be added to solution and dissolved under

ultra sonication at 35°C.The inner phase was poured into the PVA solution in water (outerphase). Following 60min of stirring, the mixture is filtered to separate then anosponges. The Nanosponges aredried in an air-heated oven at 40 °C for 12hrs.

3.Emulsion solvent diffusion method

In this method the two phases used are organic and aqueous. Aqueous phase consists of polyvinyl alcohol and organic phase include drug and polymer. After dissolving drug and polymer to suitable organic solvent, this phase is added slowly to the aqueous phase and stirred for two or more hours and then Nanosponges are collected by filtration, washed and then dried in air at room temp or in vacuum oven at 40°C for 24hours.

Polymers Used in Nanosponge Preparation:

There are various polymers and crosslinkers are used in the preparation of nanosponges, listedinTable -1 Table -1

Polymers	Copolymers	Crosslinkers
Hyper cross linked Cyclodextrines and Alkyloxycarbonyl Cyclodextrins, Methylβ-Cyclodextrin,HydroxyPropyl β-Cyclodextrins, Ethyl Cellulose.	Poly(valerolactone allylvalerolactone),Poly (valerolactone-oxepanedione),Polyvinyl alcohol.	Carbonyl diimidazoles, dianhydrides, Diarylcarbonates, Carbonate, Epichloridine, Gluteraldehyde, Pyromelliticanhydride,2,2-bis(acrylamido) Acetic acid.

Some drugsformulated as nanosponges[18]are given in table -2.

Table -2:Drugs used as Nanosponges.

Drug	**Nanosponge vehicle**	**Therapeutic indication**
Econazolenitrate	EthylCellulose Polyvinylalcohol	Antifungal
Paclitaxel	β-Cyclodextrin	Cancer
Tamoxifen	β-Cyclodextrin	BreastCancer
Resveratrol	β-Cyclodextrin	Inflammation Cardiovascular diseases Dermatitis Gonorrhea Fever Hyperlipidemia
Dexamethasone	β-Cyclodextrin	Braintumors
Temozolam ide	Poly (valerolactone-allylvalerolactone) Poly (valerolactone-allylvalerolactone-oxepanedione)	Braintumors

EVALUATIONOF NANOSPONGES

1. **Particle size determination[18]**:

The particle size can be determined by dynamic light scattering using 90 plus particle sizer equipped with MAS OPTION particle sizing software (Malvern zeta sizer). From this the mean diameter and poly dispersity index can be determined.

2. ZetaPotential[18]

Zeta potential is a measure of surface charge. It can be measured by using additional electrode in the particle size equipment.

3. LoadingEfficiency[18]

The loading efficiency of Nanosponges can be determined by the quantitative estimation of drug Loaded into Nanosponges by UV spectro photometer & HPLC methods.

4. **Microscopic study**[18]

Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) are used to study the microscopic character of nanosponge. Morphology of the Nanosponge is studied with SEM.

5. CompatibilityStudies[18]

The compatibility study of drug with the adjuvants are determined by FTIR,TLC. Crystalline characteristics can be studied by powder X-ray diffraction (XRD) and Differential Scanning Calorimetry(DSC).

6.**Thin Layer Chromatography**[18]

In TLC, formation of the complex in nanosponge is determined by the Rf values.

7.**Solubility study**[18]

The Inclusion complexation are studied by phase solubility method described by higuchi and Connors whichexamines the effect of a Nanosponge, on the solubility of drug.

8.**Single Crystal X-Ray Structure**[18]

This method used to determine the detailed inclusion structure and mode of interaction. The interaction between the host and guest molecules can be identified and the precise geometrical relationship can be established. This information obtained during the analysis lead to know about the formation of inclusion complexes.

9.**Drug release study**[18]

Release study for the drug is done with dialysis membrane using Franz diffusion cell.

10.**Drug kinetic study**[18]

To investigate the mechanism of drug release from Nanosponge the release data could be analysed using Zero order,

Firstorder, Higuchi, Peppas, Hixon-Crowell, Kopcha and Makoid-Banakar etc. models. The data can be analysed using graph pad prism software.

11.**Porosity**[19]

Porosity study is carried out to check the Nanochannels and nanocavities formed in Nanosponge. Porosity of Nanosponge is determined by Helium displacement method using Helium pynometer.

12.**Swelling and water uptake**[19]

For swellable polymers like polyamidoamine nanosponges, water uptake can be determined by soaking the prepared Nanosponges in aqueous solvent. Swelling and water up take can be calculated using equations.

13.**Resiliency(Viscoelasticproperties)**[19]

Resiliency of sponges can be modified to produce beadlets that is softer or firmer according to the needs of the final formulation. Increased crosslinking tends to slow down the rate of release. Hence resiliency of sponges will be studied and optimized as per the requirement by considering the releaseas a function of cross-linking with time.

ComparisonofSomeEffectiveVesicularSystems[19]**.**

Liposome, niosome, ethosome, transferosome and nanosponge are colloidal drug delivery systems. They all are nanometric in size. Liposome, noisome and transferosomes have some stability problems which is discuss below in table but Nanosponge enhanced the stability of drug.

Table-3:Comparison of Nanosponge with Vesicular system.

Liposome	Niosome	Ethosome	Transferosome	Nanosponge
Liposome consist of one or more concentric lipid bilayers, which enclose an internal aqueous volume.	Niosomes are non-Ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, withor without incorporation of cholesterol or other lipids.	Ethosomes are lipid vesicles containing phospholipids, alcohol(ethanol and isopropylalcohol)in relatively high concentration and water.	Transferosomes are vesicular system consisting of phosphatidyl choline and surfactants.	Nanosponge are novel class of hyper-crosslinked polymer based colloidal structures consisting of solid nanoparticles with colloidal sizes and nanosized cavities.
The composition of liposomes is phospholipids and cholesterol.	They composed of non-ionic surfactants and cholesterol.	They composed mainly of phospholipids, high concentration of ethanol and water.	They consists of phospholipids and surfactants.	They composed of polymers and cross linkers.
Stability problems: due the formation of ice crystals in liposomes, the subsequent instability of bilayers leads to the leakage of entrapped material. The oxidation of cholesterol and phospholipids also leads to the formulation instability.	Stability problems: fusion, aggregation, sedimentation and leakage on storage. The Hydrolysis of encapsulated drug.	Ethosomes has initiated a new area in vesicular research for transdermal drug delivery which can provide better skin permeation and stability than liposomes. Application of ethosomes provides the advantages such as improved entrapment and physical stability.	Stability problem: chemically unstable because of their predisposition to oxidative degradation.	Nanosponge are chemically and physically stable. They increase the stability and bioavailability, modify drug release and reduce side-effects.

APPLICATIONS OF NANOSPONGE:

- **Topical delivery[19]:**

Local anesthetics, antifungal and antibiotics are among the category of the drugs that can be easily formulated as topical Nanosponges. A wide variety of substances can be

Incorporated into a formulated product such as gel, lotion, cream, ointment, liquid, or powder.

- **Enhanced Solublity[19]**

The Nanosponge system has pores, that increase the rate of solubilisation of poorly soluble drug by entrapping such drugs in pores. Due to nanosize surface area significantly increased and increase rate of solubilisation.

- **Nanosponge as chemical sensor[19]:**

Nanosponges which are the type of " metaloxides "act as a chemical sensors which is used in highly sensitive detection of hydrogen using Nanosponge titania. Nanosponge structure initially have no point of contact so there is less hinderance to electron transport and it results in higher 3D interconnect Nanosponges titania which is sensitive to H2 gas.

- **Used as Taste masking agent.[40]**
- **Chemotherapy[19]**

The tiny sponges are filled with drug and expose a targeting Peptide that bind to radiation induced cell surface receptor on tumor. When the sponge encounter tumor cell they stick to surface and triggered to release cargo. One of the important drug formulated as Nanosponge is paclitaxel, the active ingredient in the anti-cancer therapy Taxol.

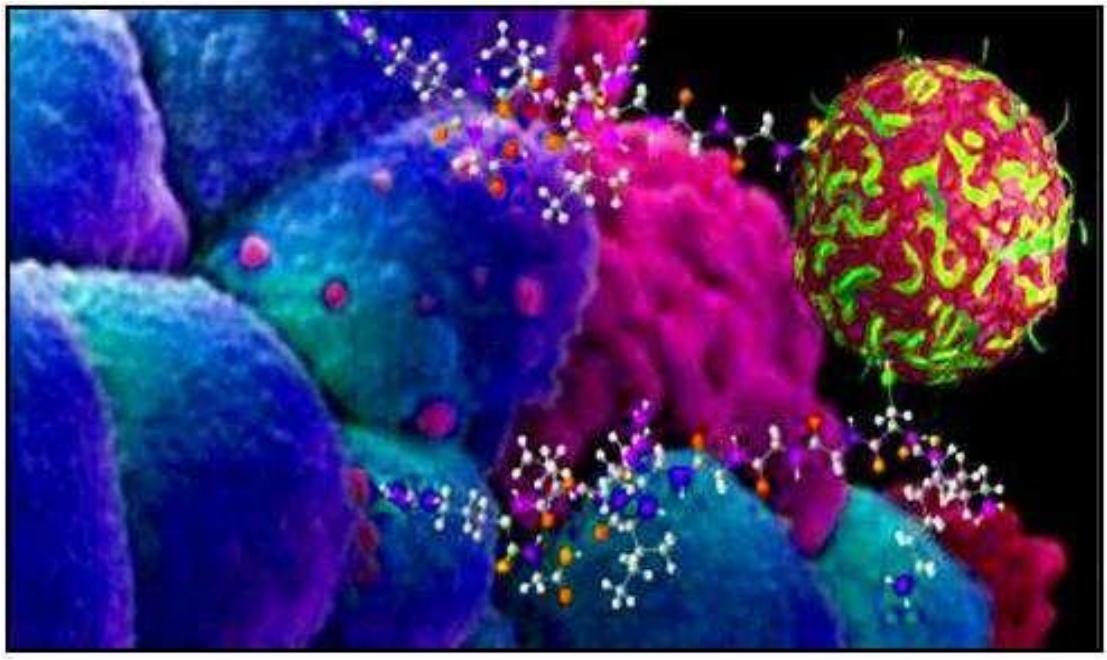


Figure-6: Nanosponge particle attaching to human breast cancer cells[16]

- **In anti viral therapy[17]:**

  Nanosponges can be useful in the ocular, nasal and pulmonary administration routes. The selective delivery of antiviral drugs or small interfering RNA (siRNA) to the nasal epithelia and lungs can be accomplished by nanocarriers in order to target viruses that infect the RTI such as respiratory sinctial virus, influenza virus and rhinovirus. They can be used for Human Immunodeficiency Virus (HIV), HepatitisB Virus (HBV) and Herpes Simplex Virus (HSV).Drugs used are zidovudine, saquinavir, interferon-α, acyclovir, nelfinavir etc.

- **In protein drug delivery[17]**

  Bovine serum albumin(BSA) protein is unstable in solution form so stored in lyophilized form. Swellable cyclodextrin based poly(amidoamino)Nanosponge enhanced the stability of proteins like BSA. Nanosponges have also been used for enzyme immobilization, protein encapsulation, and subsequent controlled delivery and stabilization.

- **In oxygen delivery system[19,54]**

  Cyclodextrin Nanosponges have also been developed as oxygen delivery system.

  Nanosponge has the ability to store and to release oxygen slowly overtime. Oxygen-filled nanosponges could supply oxygen to the hypoxic tissues which are present in various diseases.

  Characterized by using a, ß and ϒ cyclodextrins and this are suspended in water and get saturated with water. A silicone form of membrane can also be used for oxygen permeation with the help of nanosponge/ hydrogel system. They can also applied it to hypoxic tissues caused in various type of diseases.

- Toxin absorbing Nanosponges used to absorb Toxin of staphylococcus aureus which produce resistance to Methicillin[37].

- **More effectiveness than direct injection:[56]**

  Recent research suggests that nanosponge could be up to five times more effective at reducing tumor growth than direct injection. The drug delivery system is likened to be filling virus-sized sponges with an anti-cancer drug, attaching chemical linkers that bond to a receptor on the surface of tumor cells, then injecting the sponges into the body. When the sponges come into contact with a tumor cell, they either attach to the surface or are sucked into the cell, where they off-load their deadly contents in a predictable and controlled manner.

❖ **Novel flame retardants containing cyclodextrin nanosponges and phosphorus compounds to enhance EVA combustion properties[69]**

A novel flame retardant in tumescent system, aimed to improve the fire stability of ethylene vinyl acetate copolymer (EVA), has been prepared by melt blending of the copolymer and a complex of cyclodextrin nanosponge-phosphorus compounds. As compared to traditional systems, this complex which is stable in processing conditions, has the advantage that nanosponges act as both carbon sources and foam forming agents while the phosphorus compounds are able to directly generate phosphoric acid *in situ*. In this context, cyclodextrin nanosponges undergo dehydration in presence of the acid source, generating water vapour and char, and thus protecting the copolymer against combustion.

❖ **OtherApplications**

Biomedical Application,

Analytical Application,

For Hydrogen storage,

In Agriculture,

In Floriculture,

In Food Industry,

For water Purification,

For Oil Cleaning,

As Novel flame Retardants,

Against pore forming Toxins and superbug infections, and

Micro patterning of Mammalian cell.

1. **Dr Prathima srinivas *et al*** [31] formulated and Evaluated Voriconazole Nanosponges for Oral and topical Delivery as Tablets and Gel. Voriconazole Nanosponges were prepared by Emulsion Solvent Evaporation Technique with three different Polymers. Final formulations and Nanosponges were evaluated for Drug content, Entrapment efficiency, Physical parameters, FTIR Study, *In-vitro* and *in-vivo* study, and Anti microbial activity. From the above evaluation they concluded that the three polymers used were efficient carriers for Voriconazole Nanosponge showing Diffusion controlled release.
2. **Raja CH. NV. *et al*** [36] fabricated and Evaluated Ciprofloxacin loaded Nanosponges for sustained release. Five batches of Nanosponges using different proportion of Ethyl cellulose were prepared by solvent evaporation method. The Nanosponges were evaluated for Characterization by Scanning electron microscopy, Solubility study, *In-vitro* dissolution study, and Drug entrapment efficiency. Thereby they concluded that the Final batch (F5) is considered as a best entrapped and has a greater percentage drug release.
3. **Gautam Seema *et al*** [33] developed and evaluated curcumin loaded Nanosponges for colon drug delivery. Six batches of Nanosponges were prepared and zeta potential, Drug content, Drug entrapment efficiency, Surface characteristic by SEM, Particle size, FTIR, DSC, Stability studies, *In-vitro* drug release, and Kinetic models of dissolution profiles were evaluated. Thereby they concluded that curcumin and Eudragit L -100 1:3.5 (F4 batch) has found to be the optimum formulation, that has a significant improvement of curcumin performance from Nanosponges compared to pure curcumin and other formulation.
4. **P.Suresh Kumar *et al*** [37] formulated and evaluated Miconazole nitrate Loaded Nanosponges for vaginal drug delivery. Nanosponges were prepared using different ratios of beta cyclodextrin and Di phenyl carbonate. The Miconazole nitrate was loaded into the beta cyclodextrin Nanosponges by solvent evaporation technique using various solvents. Nanosuspension was prepared with the Miconazole Nanosponge. That nanosuspension is used for the preparation of Gel using Carbopol 934 as gelling agent. Both the Nanosponge and Gel were evaluated. Thereby they concluded that the

optimized formulation has good spreadability, extrudability, mucoadhesive nature and has sustained release.

5. **Renuka Sharma *et al*** [34] fabricated and Evaluated Econazole nitrate Nanosponges as topical hydrogel. Econazole Nanosponge were prepared by using various concentration of ethyl cellulose and evaluated for entrapment efficiency, *in -vitro* drug release size measurement, Rheological properties. For hydrogel the Equilibrium swelling study, viscosity analysis, texture analysis, *in-vitro* permeation study has evaluated.

6. **Fei Wang, et al**[38] formulated Hydrogel Retaining Toxin – Absorbing Nanosponges for Local Treatment of Methicillin Resistant Staphlococcus aureus infection. The Staphlococcus aureus which are resistant to the methicillin due to their toxin activity towards the drug. These type of toxins which were forming pore and constitute important bacterial virulence factors. These toxins disrupt cells by forming pores on cellular membranes and altering their permeability for bioactivity. Hybrid nanostructures like Nanosponges may has ability to absorb toxins and by reducing their effect. That can be proved by *in-vitro* α-toxin Neutralization study, Live whole body imaging of mice to study Nanosponge retention, *in-vivo* α -toxin Neutralization study and *in- vivo* Detoxification efficacy against localized MRSA infection.

7. **Gouri Shankar and Y.K. Agarwal**[40] formulated and Evaluated β -cyclodextrin Nanosponges of a poorly water soluble drug. β-cyclodextrin Nanosponge were prepared and Simavastatin was added to it. The Simvastatin Nanosponges are evaluated for the Particle size. Polydispersity, Zeta potential, Entrapment efficiency, FT-IR, DSC,XRD, Mucoadhesive strength ,*in-vitro* release study, Kinetics study, and Stability study. Higher Solubilization and prolonged release of Simvastatin Nanosponges served the purpose of synthesizing Nanosponges. Thereby they concluded that the β-cyclodextrin based Nanosponge was an effective nanocarrier for the delivery of simvastatin.

8. **Monica R. P. Rao et al**[41] developed and Evaluated the Nanosponge- based Pediatric-controlled release dry suspension of Gabapentin for reconstitution. Nanosponge of Gabapentin were formulated using β-cyclodextrin by melt method. The Nanosponge drug complex were characterized by FT-IR, DSC and PXRD as well as evaluated for taste and saturated solubility. Then it was coated with Ethyl cellulose and Eudragit RS 100. It shows the release of drug from Nanosponge was in controlled manner, as well as the taste of Gabapentin was masked. Leaching of drug is insignificant shows the stability of Nanosponge. Thereby they conclude that the Nanosponge prepared with β-cyclodextrin was an ideal nanocarrier for controlled release.

9. **Kurhe, et al**[42]describes Scaffold based drug delivery system :A Special emphasis on Nanosponge. Nanosponges were prepared to have ability to encapsulate either hydrophilic or lipophilic drug and release the drug in a controlled and predictable fashion at the target site. Thereby they concluded Nanosponges increases the drug solubility ofpoorly water soluble drugs and protects the drug from physiochemical degradations. It can be developed asdifferent dosage forms like Paretrals,aerosol, topical, tablet and capsule. Thus, Nanosponges are a Boon for targeted and site specific drug delivery system.

10. **Shastrulagari Shivani et al**[43] reviewed Nanosponge – novel emerging drug delivery system. Types of Nanosponge, advantages, Polymers and cross linker used are reviewed. Method of preparation of Nanosponge, Factors influencing Nanosponge, characterization and applications.

11. **P.Suresh Kumar. et al** [44] prepared and Evaluated Clotrimazole loaded Nanosponges containing vaginal Gels. Vaginal gel were formulated and the various evaluation are carried out for Nanosponge and for the Gel. Thereby they concluded that the Nanosponge gel prepared with HPMC showed good extrudability, homogeneity, spreadability and required diffusion rate in comparison with other formulations and selected as suitable carrier to be delivered through vaginal route at controlled rate.

12. **Satyen J. torne, et al** [45] prepared and Evaluated for Enhance oral paclitaxel bioavailability after administration of paclitaxel loaded Nanosponge. The prepared Nanosponge is evaluated for general test for Nanosponge and the pharmacokinetics of Paclitaxel through Nanosponge.The plasma concentration of paclitaxel after the oral administration were significantly higher than the Taxol at the same dose shows the increased effect than conventional dose/

13. **Marzouk M.A., et al** [46]prepared and Evaluated the Nystatin Topical formulations with various Penetration enhancers. All the general parameters are evaluated for the gel and compared with the plain gel and concluded that the penetration enhancer used Propylene glycol showed the highest effect in the amount drug permeated followed by dimethylformamide. The antifungal activity of all Nystatin gels was found to be greater than that of commercial nystatin cream.

14. **Bazigha k. Abdul Rasool, et al**[47] *in-vitro* release study of Nystatin from chitosan buccal gel. Bioadhesive Nystatin gel with chitosan as gelling agent and various enhancers. All the general tests for gel were evaluated and then the Antifungal effect of Nystatin was carried out. From the above study thy concluded that the 5% Chitosan with10% w/w of PG as solubilizing agent has best activity and in-vitro release shows the enhancement of solubility over commercial product.

15. **Reeta T.Metha, et al**[48] formulated the Liposome encapsulaed Nystatin for systemic Candidiasis. Liposomes are formulated using various lipids and reagents. That liposomes are subjected for all the tests and concluded that the formation of liposomes reduces the toxicity and improve the release.

16. **Rodino S, et al**[49] Nystatin Determined quantitatively by spectrometric method. The average Nystatin content resulting from the five determination differs from the expected value not more than 3.15% of the average. Thereby they concluded that the analytical method based on exploiting photosensitivity and selective photo transformation of the active substance proved to be reliable for analytical control.

17. **Gabriela Badea, et al**[50] formulated Nystatin loaded nanostructured carriers for the influence of basil oil extract on the antioxidant and antifungal activity. Lipid nanoparticles were prepared with basil oil and evaluated for the anti oxidant activity and anti microbial activity. Charcterization of size , uniformity, and stability of the nanoparticle is measured. The antioxidant activity was maintained in the area of a high ability scavenge free radicals. Thereby they concluded that the NLC based on 2% basil oil and loaded with 1%nystatin was found to be the most active against Candida albicans.

18. **Sachin S. Salunkhe ,et al**[51] investigated Nystatin loaded Liposomal gel for topical application by factorial design. Nystatin liposomes were prepared by ethanol injection method and the effect of variables , size distribution , zeta potential, entrapment efficiency, and Skin permeation and drug deposition study were done. For the liposomal gel, the rheological study, drug content, content uniformity and stability studies were performed. The results were obtained by factorial design and concluded that the liposomal gel were found to be significantly increase the skin permeation and deposition in comparison to standard reference product, and has potential application in topical delivery.

19. **Ruba Kello,**[52] prepared Polyethylene Glycol Novel based Nystatin and Triamcinolone acetonide ointment. Prepared ointment is subjected to Stability studies, and they concluded that it polyethylene glycol 1500 and polyethylene glycol 400 can be used as an alternative ointment base for Nystatin and Triamcinolone acetonide.

20. **Tahseen h. Nasti, et al**[77] prepared and evaluated the pH-sensitive Nystatin liposomes against Crytococcus neoformans in murine model. The pH sensitive Nystatin liposomes were prepared by Egg Phosphatidylcholine, cholesterol and mixing the DOPE and CHEMS in the ratio of 2:3.all the ingredients were mixed in a round bottomed flask and minimum quantity of chloroform or methanol (1:1) . Solvents were carefully evaporated to for liposomes. Antifungal susceptibility test were carried out in mice.Results were compared with Nystatin. As they concluded that the infection was subsided owing to the use of pHsensitive Nystatin liposomes that release substantial drug at the pH favorable

for Cryptococcus neoformans. Nystatin , toxic in free form, can be made more effective and safe entrapping it within pHsensitive liposomes for treatment of fungal disease.

21. **Mohammad Barzegar-Jalali,et al**[53]formulated and evaluated Nystatin suspensions to determine Effect of different buffered and unbuffered dispersion medium on the physical and chemical stability. The Prepared nystatin suspensions were evaluated for the Sedimantation volume, resuspendability, Freeze-thaw cycling test, chemical testing like Arrhenius accelerated stability test and shelf life. Thereby they concluded that the buffered cellulosic suspending agent containing nystatin suspension gave stable suspension when compared to unbuffered vehicle.

22. **Kunikazu Mobibe, et al**[54] formulated and evaluated the Nystatin liposomes for the Encapsulation characteristics by effects of cholesterol and polyethylene glycol derivatives. The prepared liposomes are evaluated for the Fluoresence measurement of nystatin binding. They concluded that the encapsulation of liposomes in relation to the incorporation of cholesterol and DSPG-PEG, High encapsulation efficiency was obtained in CH-free PEG-liposomes. Molecular interaction between nystatin and DSPE-PEG appears to invovlve in the interaction of amino group of nystatin with the phosphate group of nystatin in the liposomal membrane. These results may help in the development of effective pharmaceutical formulations for injectable hydrophobic drugs with reduced side effects.

23. **Akhani Jolly R*, et al**[59] formulated developed and evaluated the in situ gel for vaginal drug delivery of anti fungal drug. When poloxamers PF 127 and PF 68) were used in combination for developing thermosensitive and mucoadhesive *in situ* gel, low to moderate amounts of HPMC K4M and PF68 were to be used to achieve the desired gelation temperature, gel strength, mucoadhesion, drug release profile and viscosity required for a sustained vaginal drug delivery system of nystatin. It was concluded that the amounts of HPMC K4M had a significant effect on bioadhesion force and gel strength of the formulated thermosensitive and mucoadhesive in situ gel. The quadratic mathematical model developed is applicable to predicting ***in situ*** gel vaginal formulations with desired characteristics.

24. **Rawia M. Khalil* et al**[66] formulated and characterized the nystatin-loaded nanostructured lipid carriers for topical delivery against cutaneous candidiasis . Nys NLC is prepared with various surfactants, and Lipids. The prepared NLC were evaluated for Particle size, Entrapment, TEM, DSC, Stability and Release , antimicrobial activity. Thereby they conclude that the prepared Nys NLC is better than the commercial products.

25. **Kunikazu Moribe, et al**[67] investigated the molecular state of Nystatin encapsulated in liposomes by spectrometrically. Liposomes with DPPC, DPPC/CH, DPPC/DSPE-PEG, DPPC/CH/DSPE-PEG, DPPC/CH/DSPE-PEG. The molecular state of Nystatin in liposomes are subjected to spectrometically, thereby they concluded that compared with AmB, Nystatin does not readily form a complex with DSPE-PEG and the enhanced encapsulation of Nystatin would be attributed to the CH-free liposomes. DPPC liposomes shows the least stability in these composition.

26. **A. Hassan, et al**[68] confirmed Candida albicans endogenous fungal endophthalmitis in a patient with chronic Candidiasis. They concluded that confirmed C. albicans endophthalmitis in a patient with chronic vaginal Candidiasis, and need a particular importance in patients with additional immunosuppressive risk factors. So need to carefully monitor patient's eyes or administer antifungal prophylaxis in patients with active genital Candidiasis undergoing procedures. In this case conventional microbiological laboratory diagnostics were negative and EFE was only confirmed with molecular techniques. This highlights the need to utilize DNA diagnostics in the early identifications of at risk patients.

27. Journal of Antimicrobial Chemotherapy[69] studied the use of fluconazole and itraconazole in the treatment of Candida albicans. In this article they concluded that fluconazole remains a first-line antifungal agent of choice for the treatment of *C.* albicans infections, because of its well-known efficacy and safety profile;

its suitability for use in children, the elderly and patients with impaired immunity; its range of formulations; and its cost.

28. **Mary K. Truhlar,et al** [69] quantified the antifungal activity of Nystatin incorporated in denture liners by new assay technique. Preparation incorporating higher concentrations of nystatin were more potent inhibitor of Candida albicans growth, and loss of antifungal activity for all concentrationswas the greatest from day 0 to 2.Incorporating a single doseof an antimycotic agents into the reline material of the denturethat allows release of the agent over 2 week period is a treatment modality that offers considreable promise for the management of oral candidiasis.

29. **B.Indira, et al**[72] reviewed Nanosponges, a New Era in Drug Delivery. They Reviewed on the Nanosponge about the Advantages, Disadvantages, Method of Preparation, Polymers and crosslinkers Used, Factors infiuencing Nanosponge, Characterization of Nanosponges, and their applications. They concluded that this new invention, nanosponges will pave a way in overcoming the challenges in designing of targeted drug delivery systems because of their ability to accommodate either hydrophilic or lipophilic drugs and release them in a controlled and predictable manner at specific site in the body. The release rate can be modulated by controlling the polymer and cross linker ratio. The nanosponges have been found to possess a profound ability to protect essential biomarkers in diseases (cancer for example) and biocatalysts from physicochemical degradation. In near future they stand as milestones in drug delivery.

30. **E. K.Patel et al**[73] reviewed Nanosponge and micro sponges a Novel drug delivery system. They reviewed about all the aspect of Nanosponge and elaborated about the uses of prepared Nanosponges. **Camptothecin (CAM),** a plant alkaloid and potent antitumor agent, has a limited therapeutic utility because of its poor aqueous solubility, lactone ring instability and serious side effects. Cyclodextrin based nanosponges(NS) are a novel class of cross-linked

derivatives of cyclodextrins. They have been used to increase the solubility of poorly soluble actives, to protect the labile groups and control the release. This study aimed at formulating complexes of Camptothecin with ßcyclodextrin based nanosponge. They concluded that the Nanosponges are a Nanosized particle entrap wide variety of products, enhance solubility, provide prolonged use and provide patient compliance with the formulation.

AIM OF WORK

The aim of the present study is

- To formulate Nystatin loaded Nanosponge using various polymers.
- To provide an efficient dosage form.

PLAN OF WORK

The present work is designed and planned as follows:

- Drug-polymer compatibility studies by FT-IR.
- Determination of λ_{max} of Nystatin.
- Preparation of Calibration curve of Nystatin.
- **Formulation of Nystatin loaded Nanosponge**
  - Formulation of Nystatin Nanosponge using different polymers (Ethyl cellulose and Polymethyl methacrylate) at different ratios (Drug:Polymer-1:1,1:2,1:3,1:4and 1:5) using Solvent Emulsion evaporation Method.
- **Characterization of Nystatin loaded Nanosponges**
  - Determination of physical properties.
  - Determination of drug content.
  - Determination entrapment efficiency.
  - Determination of particle size distribution.
  - i*n-vitro* release studies of Nystatin loaded Nanosponge.
  - Kinetics of drug release.
  - Morphology of Nanosponge by Scanning electron microscopy(SEM) technique.
  - Stability studies.
- **Formulation of Nystatin loaded Nanosponge Gel for optimized product:**
- **Evaluation of Nystatin loaded Nanosponge Gel.**
  - Determination of physical properties.
  - Determination of drug content.
  - *In-vitro* release studies of Nystatin loaded Nanosponge Gel.
  - Antifungal activity of the Nystatin loaded Nanosponge Gel.

RATIONALE OF THE STUDY

Fungal Infections have been increasing in incidence in the human population over the last 15 years or so, largely as a consequence of increased numbers of cancer and immune compromised patients, who are at risk owing to weakened immune systems and chronic nature of the disease.

Systemic fungal infections are more serious as they are usually more difficult to diagnose, are chronic in nature, and in some cases, can become life-threatening. They occur more frequently in individuals with compromised immune systems (AIDS patients; transplant patients; cancer patients). Prophylactic treatment is sometimes indicated in AIDS patients and bone marrow transplant patients, but risk of developing resistance is high. Life-threatening infections require the use of more potent but much more toxic antifungal.

Superficial fungal infections are almost always caused by dermatophytes or yeasts. In some instances, they can be rather tenacious, requiring very long treatments, sometimes with both oral and topical drugs.

RATIONALE OF SELECTION OF DRUG[61]

Nystatin (Nys) is a polyene antifungal antibiotic, one of the oldest antifungal drug, produced by Streptomyces noursei strains Nys monomers selectively interact with ergosterol causing membrane disruption and eventual cell death. Nys possesses a broad spectrum with both antifungal and fungistatic activity, being effective against azole-resistant strains of Candida and amphotericin B resistant strains of Candida albicans. Nys is indicated for treatment of Cutaneous and mucocutaneous fungal infections caused by Candida species, the main yeast capable of infecting the oral mucosa, C. albicans being the most common species isolated . Oral Candidiasis is not a lethal disease in healthy patients. It is mainly caused by antibiotic or corticosteroid treatment and dental prosthesis. However, it must be treated to avoid chronic and systemic invasions of other tissues, especially among patients with diabetes mellitus, immune compromised or under aggressive treatments (e.g. chemotherapy) to prevent opportunistic invasive fungal infections.

The presence of a large lactone ring with several double bonds renders it an amphiphilic and amphoteric molecule with poor solubility in aqueous media (360 mg/L at

24° C), which reveals great formulation challenges. This fact support that an increasingly important area of pharmaceutical research is focused on finding safe and effective methods of solubilizing poorly soluble drugs.

Adverse effect of Nys is very rare. From Neonates to Elderly can use Nys, even Pregnant women can use it safely.[62] Cost of Nystatin cream when compared to other antifungals is low.

RATIONALE FOR THE SELECTION OF DOSAGE FORM.[20,65]

Nanosponge are mainly for **Topical drug delivery.** It is very much suitable for antifungal agents. Nanosponge is a nano sized particle, has larger surface area, thereby the penetration is high when compared to other conventional products. Low solubility drugs formulated as Nanosponge may increase the solubility thereby increase the release and activity. **Stability** of Nanosponge is higher than most of the novel formulation.

Nystatin has only topical action, there is no systemic effect, and it is slightly soluble in water, hence preparations as Nanosponge increases its effect by increasing solubility.

CANDIDA SPECIES

Candida species is the most common cause of opportunistic fungal infection worldwide. *Candida* is the major fungal pathogen of humans, causing diseases ranging from superficial mucosal infections to disseminated, systemic infections that are often life threatening. A striking feature of its pathogenicity is ability to grow in yeast, pseudohyphal and hyphal forms. The hyphal form has an important role in causing disease by invading epithelial cells and causing tissue damage. Among *Candida* spp., *Candida albicans* is the most common infectious agent. This dimorphic yeast is a commensal that colonizes skin, the gastrointestinal and the reproductive tracts. Non-*C. albicans* species are also emerging pathogens and can also colonize human mucocutaneous surfaces. The pathogenesis and prognosis of candidial infections are affected by the host immune status and also differ greatly according to disease presentations. Candidiasis is also technically known as candidosis, moniliasis and oidiomycosis[22].

Table-4 : Candida species implicated in human infections.

Common species	Less common species	Rare species
Candida albicans Candida glabrata	Candida dubliniensis Candida famata	Candida blankii Candida bracarensis
Candida tropicalis Candida parapsilosis Candida krusei	Candida inconspicua Candida lipolytica Candida metapsilosis	Candida catenulate Candida chiropterorum Candida ciferri
Candida guilliermondii Candida lusitaniae	Candida norvegensis Candida orthopsilosis	Candida eremophila Candida fabianii
Candida kefyr	Candida pelliculosa Candida rugosa	Candida fermentati Candida freyschussii
	Candida zeylanoides	Candida haemulonii Candida intermedia
		Candida lambica Candida magnolia
		Candida membranae faciens Candida nivariensis Candida palmioleophila
		Candida pararugosa Candida pseudohaemulonii
		Candida pseudorugosa Candida pintolopesii
		Candida utilis Candida valida
		Candida viswanathii

Candida albicans

It is a diploid yeast with two pairs of 8 chromosomes. Its genome size is about 16 Mb. It possesses 6,159 coding genes. It is the predominant cause of invasive fungal infections. People at risk include those suffereing from HIV, cancer and intensive care unit patients for example those undergoing major surgery and organ transplants.

Candida glabrata (Torulopsis glabrata)

It is a small, haploid, monomorphic yeast with 13 chromosomes and a genome size of 12.3 Mb. It possesses 5283 coding genes. It has become important because of its increasing incidence worldwide and decreased susceptibility to antifungals. Its emergence is largely due to an increased immunocompromised patient population and widespread use of antifungal drugs. In many hospitals, *C. glabrata* is the second most common cause of candidemia.

Candida tropicalis

It is a diploid, with 10 to 12 chromosomes and a haploid genome size of 15 Mb. It possesses 6258 genes. *C. tropicalis* is the third or fourth most commonly recovered *Candida* species from blood cultures. *C. tropicalis* has progressively been observed to be the commonest cause of invasive candidiasis in neutropenic patients such as those with acute leukaemia or those who have under- gone bone marrow transplantation.

Candida parapsilosis

It is a diploid or aneuploid with 14 chromosomes and a genome size of 16 Mb. It possesses 5733 genes. It is one of the principal causes of invasive Candidiasis. In most parts of the world, it is the third most common cause of candidemia especially in patients with intravenous cathe- ters, prosthetic devices, and intravenous drug use. It is one of the most common causes of candidemia in neonatal intensive care units.

Candida krusei

It is a diploid, with 3-5 chromosomes and genome size of 11 Mb. It is the fifth most common bloodstream isolate, although, less common (1 to 2%), *C. krusei* is of clinical significance because of its intrinsic resistance to fluconazole and reduced susceptibility to most other antifungal drugs. It is frequently recovered from patients with hematological malignancies complicated by neutropenia tends to be associated with higher mortality rates (49 vs 28% with *C. albicans*), and lower response rates (51 vs 69% with *C. albicans*..

Candida guilliermondii (teleomorph-Pichia guilliermondii)

It is a haploid. It is reported to have genome size of 12 Mb and possess coding genes 5920. It has been isolated from environmental surfaces and from the skin and nails of healthcare workers. It has been shown to cause hematogenously disseminated Candidiasis.

Candida lusitaniae (teleomorph-Clavispora lusitaniae)

It is a haploid. It has genome size of 16 Mb. Among, the rare non-*albicans Candida* species, it has emerged during the last 20 years as an important nosocomial pathogen throughout the globe.

Candida kefyr

It consists of 18 chromosomes. *Candida kefyr* was only isolated from urine cultures (Weichert et al., 2012). It has been reported to cause systemic *Candida* infection in patients with neutropenic leukemia and in a woman with underlying heart disease. Very recently, it has been described as a pathogen causing invasive fungal enteritis in a patient with underlying haematological disease following bone marrow transplantation.

Major types of Candidiasis

Candidasis is an acute or chronic infection produced by *Candid*a, generally limited to the skin and mucous membranes, but it could produce a serious systemic disease.

Mucosal candidiasis

Candidal infections are restricted to non-sterile mucosal surface for example oropharyngeal and vulvuvaginal Candidiasis.

- **Oropharyngeal candidiasis (OPC)**

Oral candidiasis is one of the most common, oral mucosal infections seen in persons with HIV. *Candida* is commensal organism and part of normal oral flora in about 30 to 50% of the population. There are three general factors that may lead to clinically evident oral candidiasis : immune status of host, oral mucosal environment and particular strain of *C. albicans* (hyphal form is usually associated with pathogenic infection).

The ability of the yeasts to overcome host clearance mechanisms and to colonize surfaces can be considered as a risk factor for oral infection. The balance between *Candida* colonization

and candidiasis relies on the balance between pathogen characteristics (e.g. production of adhesins, secreted aspartylproteinases) and host factors.

Figure-7 : Oral Candidiasis[22].

Figure-8 : Cutaneous Candidiasis[27]

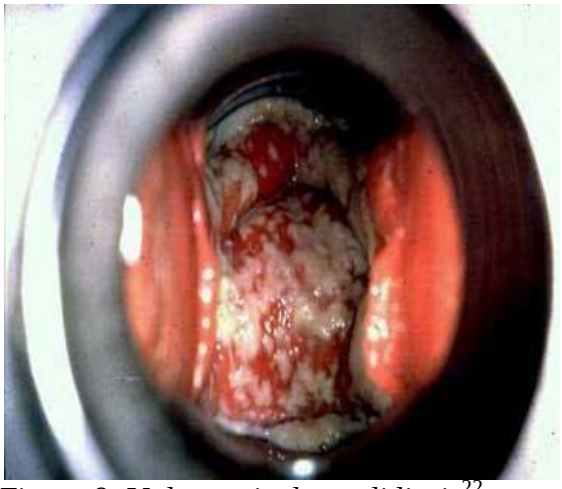


Figure-9 :Vulvovaginal candidiasis[22]

Figure-10 : Congenital Cutaneous candidiasis[27].

Vulvovaginal candidiasis (VVC)

Vaginal candidiasis is the most frequent reason for gynecology consultation in primary health care services. Disease is usually associated with considerable morbidity, healthcare cost, discomfort, pain and sexual functioning; however, it is seldom life threatening. The symptoms associated with VVC are eczematoid dermatitis lesions that

sometimes show vesicular and grey-white psuedomembrane, vulval pruritis, burning, erythema and curd like discharge.

Cutaneous candidiasis

Cutaneous candidiasis is usually secondary infection of skin and nail (body folds) in predisposed patients. It occurs as a sub-acute or chronic infection. Disease involvement may be localised or generalised to the skin or nails. The spectrum of cutaneous candidiasis includes diaper rash, intertrigo candidiasis, *Candida* folliculitis, Otomycosis, Onychia and Paronychia. It usually occurs in warm, moist and creased area, such as axillary folds, inguinal or intergluteal areas. It is fairly common opportunistic disease and usually lead to maceration and trauma in skin. It is commonly found in diabetics and obese people. Other predisposing factors are antibiotic and oral contraceptives become macerated.

Invasive candidiasis

Invasive infections can involve virtually any organ. *C. albicans* continue to account for the majority of invasive fungal infections, there has been a recent increase in disease due to non-*albicans Candida* species (and antifungal-resistant *Candida* isolates).

Systemic or disseminated candidiasis

Severe organ invasive or systemic hematogenously disseminated candidiasis is characterized by spreading of the *Candida* cells into almost the entire body with a tendency to create abscesses in vitally important organs, inducing their failure which leads to mortality in ~50% of all cases, irrespective of administration of intensive antifungal therapy.

Risk factors for mucocutaneous candidiasis may include[24]:

I. Cutaneous candidiasis
   a. Diabetes mellitus
   b. Tropical environment
   c. Obesity
   d. Use of systemic corticosteroids or anti- biotic therapy
   e. Neutropenia
   f. HIV infection
   g. Other immunocompromised states

h. Occlusion (e.g., diapers, casts. Dressings)
i. Diseases which disturb the integument(e.g., psoriasis. contact dermatitis)
j. Other
2. Oral candidiasis
a. Use of broad-spectrum antibiotics systemic, topical, and inhalational cortico steroids; and cytotoxic drugs
b. Radiation therapy
c. HIV Infection
d. Other immunocompromised states
e. Age (i.e, infants and elderly)
f. Occlusion (e.g., dentures)
g. Other

Diagnosis[23]

A. Clinical
I. History may include
a. General medical history, especially but not limited to:
1. History of weight loss or weight gain
2. Endocrine diabetes mellitus
3. Risk factors for HIV disease
4. Presence of other risk factors.
5. Use of systemic medications
6. Other
b. Sexual practices
c. Recurrent infections
d. Duration of condition
e. Current treatment(s) topical and systemic of
1. Mucocutaneous candidiasis
2. Other disease
f. Past treatment(s) topical and systemic of
1. Mucocutaneous candidiasis
2 Other disease
g. Family history of diabetes mellitus and/or mucocutaneous candidiasis
h. Occupation
i. Dermatophytic infection, particularly tinea pedis, tinea cruris
j. Drug allergies
k. Other
II. Physical examination may include
a. General physical examination as indicated
b. Examination of the involved area, but special attention may be directed to
1) Skin folds
2) Diaper area

3) Comers of mouth and mucous membranes (e.g., perleche)
4) lnterdigital spaces
5) Scrotum, glans penis and foreskin, crural folds, gluteal area
6) Perianal area
7) Vagina, vulva
8) Axillae
9) Nail unit
10) Other

c. Extent of involvement
d. Clinical appearance
e. Associated findings
1) Secondary bacterial infection
2) Postinflammatory hyperpigmenta- tion and hypopigmentation
3) Onychodystrophy and onycholysis
4) Excoriations
5) Other
f. Other

B. Diagnostic tests

l. Potassium hydroxide preparation (KOH)

Materials is obtained from the site of infection. If the lesion is a pustule, the purulent material can be used for the specimen. In cases of cutaneous involvement, the specimen can be obtained from the edge of a lesion. The material is placed on a glass slide and 10% to 20% KOH is added with or without dimethyl sulfoxide. A fungal stain, such as Chlorazol Black E, or Parker's blue black ink may be used to highlight the pseudohyphae. The presence of pseudohyphae and yeast forms confirm infection

2. Fungal culture

Candida species are yeasts rather than molds and therefore grow as yeast colonies on Sabouraud's glucose agar. The addition of cycloheximide to Sabouraud's agar may inhibit many species of *Candida* and other saprophytes. However, *C. albicans* will grow on media containing cycloheximide. Examples of such media include Mycosel, Mycobiotic, and dermatophyte test medium. Although *C. albicans* will grow on dermatophyte test media, the agar will not exhibit a red color change as occurs with dermatophyte growth if read at the appropriate time interval, as indicated by the manufacturer's instructions.

TREATMENT[22,24]

Oral Candidiasis

Topical azoles (clotrimazole troches),

Oral azoles (fluconazole, ketoconazole, or itraconazole), or

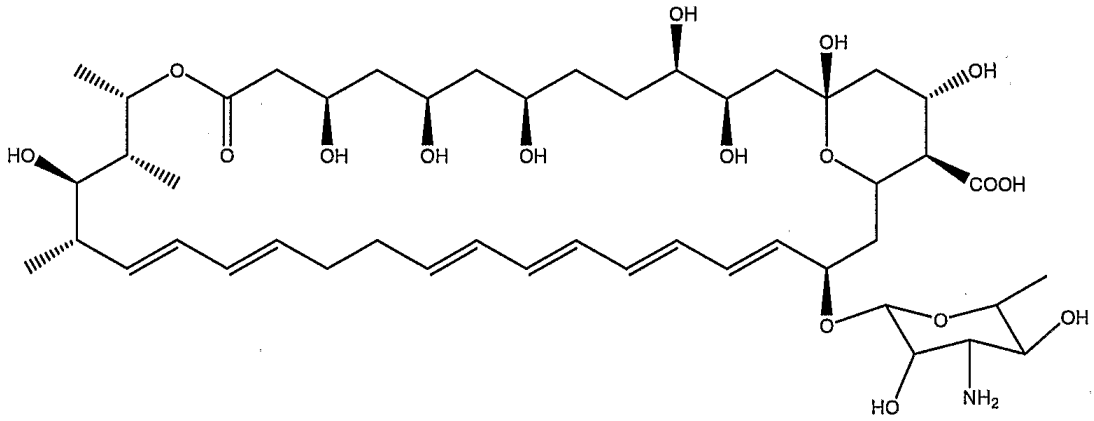
Oral polyenes (such as nystatin (oral candidiasis in infants, Nystatin suspension [100, 000 units/ml] dropped into the mouth at 4-6 hour intervals or after each feed is usually used) or oral amphotericin B).

Vulvovaginal candidiasis

Topical agents including azoles (all are used for 1 to 7 days depending on risk classification: over-the counter (OTC) clotrimazole, OTC butoconazole, OTC miconazole, OTC tioconazole, terconazole), nystatin [100,000 U per day for 7 to 14 days], oral azoles (ketoconazole (400 mg for five days), which is not approved in the United States); itraconazole (200 mg for 1 day, or 200 mg per day for three days), which is not approved in the United States; and fluconazole (150 mg).

NYSTATIN

Structural formula



CAS Number[61]

1400-61-9

Synonyms[25]

Mycostatin, Nistatina,NYS,Nystatin, Nystatine and Nystatinum.

Empirical formula[25]

$C_{47}H_{75}NO_{17}$

Chemical name[25]

(21E,23E,25E,27E,31E,33E)-20-{[(3S,4S,5S,6R)-4-amino-3,5-dihydroxy-6-methyloxan-2-yl]oxy}-4,6,8,11,12,16,18,36-octahydroxy-35,37,38-trimethyl-2,14-dioxo-1-oxacyclooctatriaconta-21,23,25,27,31,33-hexaene-17-carboxylic acid

Molecular weight[61] : 926.1

Description[61,62]

Nature **:** A Yellow to slightly brown powder, Hygroscopic.

Odour : Characteristic.

Solubility : Freely soluble in Dimethylformamide and Dimethyl sulphoxide. Slightly soluble in Methanol,Very slightly soluble in water, insoluble in chloroform, in ether, and in Ethanol (95%).

pH : 6.5 to 8, determined in a 3.0 percent w/v.

Melting point : 130˚C

Mechanism of action[25]

Nystatin exerts its antifungal activity by binding to ergosterol found in fungal cell membranes. Binding to ergosterol causes the formation of pores in the membrane. Potassium and other cellular constituents leak from the pores causing cell death. The figure :shows the Mechanism of action Nystatin with the example of Amphotericin B.

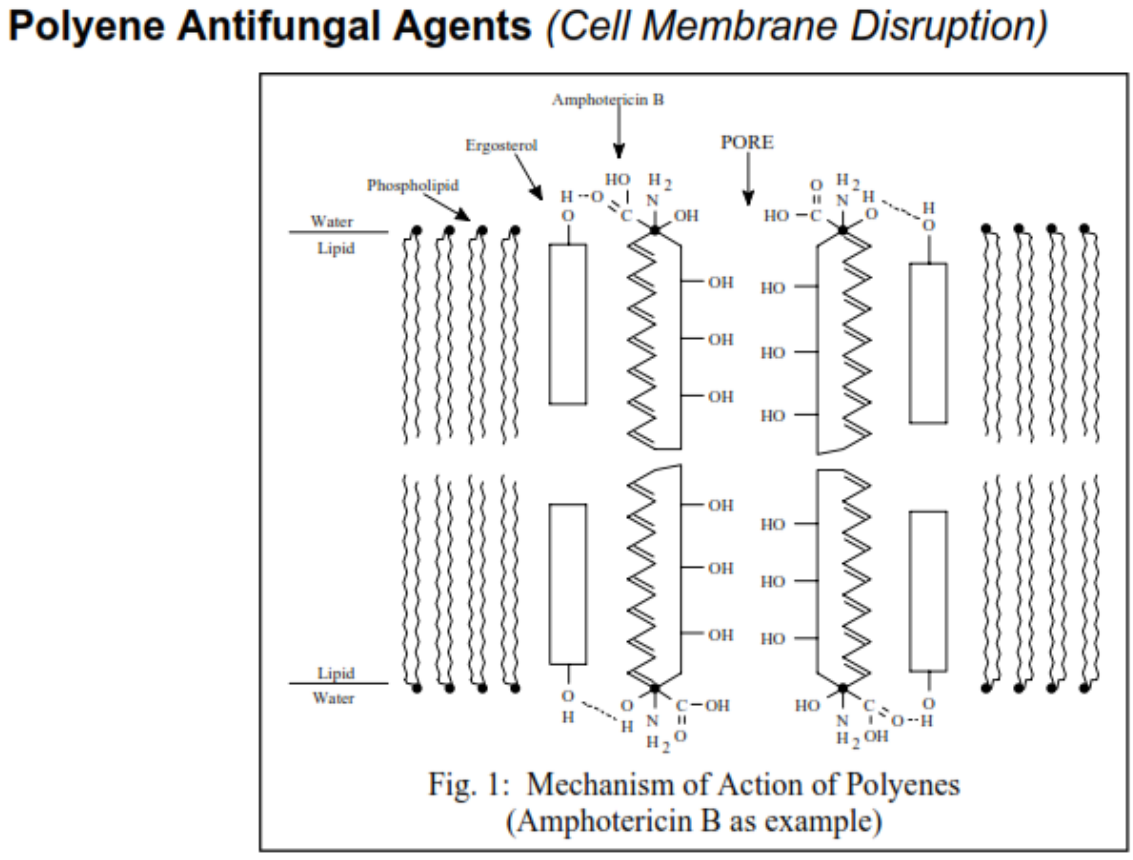


Figure-11: Mechanism of action.[57]

Pharmacokinetics[26]

Gastrointestinal absorption of Nystatin is insignificant. Most orally administered Nystatin is passed unchanged in the stool. In patients with renal insufficiency receiving oral therapy with conventional dosage forms, significant plasma concentrations of Nystatin may occasionally occur. Onset of action is rapid, Unknown peak and Half-life, Duration of action for 6-12 hours.[59]

Therapeutic indications[26]

Nystatin(topical powder, cream and ointment) is indicated in the treatment of cutaneous or mucocutaneous mycotic infections caused by *Candida albicans* and other susceptible *Candida* species.

Dosage and administration[26]

- **Usual Adult Dose for Cutaneous Candidiasis**

Apply nystatin topical cream, ointment or powder in a quantity sufficient to cover the affected area and immediately surrounding skin 2 to 4 times a day.

The powder formulation is useful for the treatment of moist areas or lesions.

Therapy should be continued for 2 to 8 weeks, depending on the nature and severity of the infection.

- **Usual Adult Dose for Vaginal Candidiasis**

Insert one Nystatin vaginal tablet (100,000 units) vaginally once a day, preferably at bedtime, for a total of 14 days.

Usual Pediatric Dose for Cutaneous Candidiasis

Apply Nystatin topical cream, ointment or powder in a quantity sufficient to cover the affected area and immediately surrounding skin 2 to 4 times a day.

The powder formulation is useful for the treatment of moist areas or lesions.

Therapy should be continued for 2 to 8 weeks, depending on the nature and severity of the infection.

Usual Pediatric Dose for Vaginal Candidiasis

>13 <18 years: Insert one nystatin vaginal tablet (100,000 units) vaginally once a day, preferably at bedtime, for a total of 14 days.

Other Comments

For fungal infections of the feet caused by Candida, dust the powder freely on the feet as well as in shoes and socks.
The vaginal tablets should be used continuously, regardless of initiation of menses.
For external use only. Avoid contact with the eyes.

Contraindication[26]

The preparation is contraindicated in patients with a history of hypersensitivity to any of its components.

Drug interactions[26]

Minor drug interaction with Vitamins only

Side effects[26]

Nystatin is well tolerated even with prolonged therapy. Oral irritation and sensitization have been reported.

Nystatin vaginal tablets have included vaginal irritation, burning, and itching. At least one case of severe vulvovaginitis has also been reported.

Gastrointestinal: Diarrhea (including one case of bloody diarrhea), nausea, vomiting, gastrointestinal upset/disturbances.

Dermatologic: Rash, including urticaria , Skin irritation, burning, itching, eczema, and pain on application in less than 0.1% of patients. Stevens-Johnson syndrome has been reported very rarely.

Other: Tachycardia, bronchospasm, facial swelling, and non-specific myalgia have also been rarely reported.

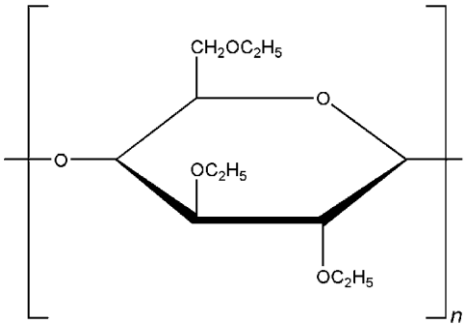
Hypersensitivity[26] Hypersensitivity side effects associated with Nystatin topical powder, cream, and ointment have included allergic reactions and allergic contact dermatitis in less than 0.1% of patients.

Storage[62]

In an airtight container , protected from light.

ETHYL CELLULOSE[79]

Structural formula



Nonproprietary names

BP –Ethylcellulose

PhEur -Ethylcellulose

USP-NF-Ethylcellulose

Synonyms

Aquacoat ECD; Aqualon; Ashacel; E462; Ethocel; ethylcellulosum;Surelease.

Empirical formula

$C_{12}H_{23}O_6(C_{12}H_{22}O_5)nC_{12}H_{23}O_5$.

Chemical name

Cellulose ethyl ether

CAS Number

[9004-57-3]

Molecular weight

Description

Ethylcellulose is a tasteless, free-flowing, white to light tan-colored powder.

Solubility

Ethylcellulose is practically insoluble in glycerin, propylene glycol, and water.

Ethylcellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform,methyl acetate, and tetrahydrofuran, and in mixtures ofaromatic hydrocarbons with ethanol (95%).

Ethylcellulose that contains not less than 46.5% of ethoxyl groups is freelysoluble in chloroform, ethanol (95%), ethyl acetate, methanol,and toluene.

Stability and Storage Conditions

Ethylcellulose is a stable, slightly hygroscopic material. It ischemically resistant to alkalis, both dilute and concentrated, andto salt solutions, although it is more sensitive to acidic materialsthan are cellulose esters.

Ethylcellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. This may beprevented by the use of antioxidant and chemical additives thatabsorb light in the 230–340 nm range.

Ethylcellulose should be stored at a temperature not exceeding32˚C (90˚F) in a dry area away from all sources of heat. It shouldnot be stored next to peroxides or other oxidizing agents.

Incompatibility

Incompatible with paraffin wax and microcrystalline wax.

Application

Ethylcellulose is widely used in oral and topical pharmaceutical

Hydrophobic coating agent for tablets and granules.

High-viscosity grades of ethylcellulose are used in drug microencapsulation.

Used as Binder

Ethylcellulose is used as a thickening agent in creams, lotions, or gels

Ethylcellulose has been studied as a stabilizer for emulsions.

Microencapsulation 10.0–20.0 %

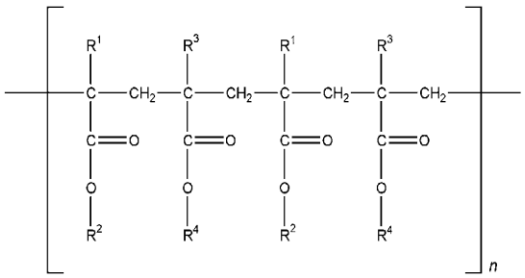
Sustained-release tablet coating 3.0–20.0%

Tablet coating 1.0–3.0 %

Tablet granulation 1.0–3.0%

POLYMETHYL METHACRYLATE[79]

Structural formula



Synonyms

Methyl methacrylate polymer,PMMA.

Empirical formula

$(C_5H_8O_2)n$

Description

Transparent Crystals, odourless.

Functional Category

Film-forming agent, tablet binder, tablet diluent.

Application

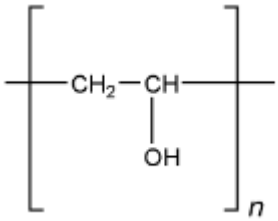
Depending upon the ratios use may vary

- Polymethacrylates are primarily used in oral capsule and tablet formulations as film-coating agents
- Eudragit RL, RS, NE 30 D, NE 40 D, and NM 30 D are used toform water-insoluble film coats for sustained release products.
- Solid polymers may be used in direct-compression processes in quantities of 10–50%.

- Polymethacrylate polymers may additionally be used to form the matrix layers of transdermal delivery systems and have also beenused to prepare novel gel formulations for rectal administration.

POLYVINYL ALCOHOL[79]

Structure



Synonyms

Airvol; Alcotex; Elvanol; Gelvatol; Gohsenol; Lemol; Mowiol,Polyvinol; PVA; vinyl alcohol polymer.

Empirical formula

$(C_2H_4O)_n$

Molecular weight

Commercially available grades of polyvinyl acohol.

High viscosity -200 000
Medium viscosity -130 000
Low viscosity -20 000

Functional category

Coating agent; lubricant; stabilizing agent; viscosity-increasingagent.

Description

Polyvinyl alcohol occurs as an odorless, white to cream-coloredgranular powder.

Properties

Melting point: 228˚C for fully hydrolyzed grades;
180–190˚C for partially hydrolyzed grades.

Solubility : soluble in water; slightly soluble in ethanol (95%);insoluble in organic

solvents. Dissolution requires dispersion (wetting) of the solid in water at room temperaturefollowed by heating the mixture to about 90˚C for approximately 5 minutes. Mixing should be continuedwhile the heated solution is cooled to room temperature.

Stability andstorage conditions

- Polyvinyl alcohol is stable when stored in a tightly sealedcontainer in a cool, dry place.
- Aqueous solutions are stable incorrosion-resistant sealed containers. Preservatives maybeadded to the solution if extended storage is required.
- Polyvinyl alcohol undergoes slow degradation at 100˚C and rapid degradation at 200˚C; it is stable on exposure to light.

Safety

Polyvinyl alcohol is generally considered a nontoxic material. Itis nonirritant to the skin and eyes at concentrations up to 10%;concentrations up to 7% are used in cosmetics.

Regulatory status

Included in the FDA Inactive Ingredients Guide (ophthalmicpreparations and oral tablets).Included in nonparenteralmedicines licensed in the UK.Included in the Canadian List of Acceptable Non-medicinal Ingredients.

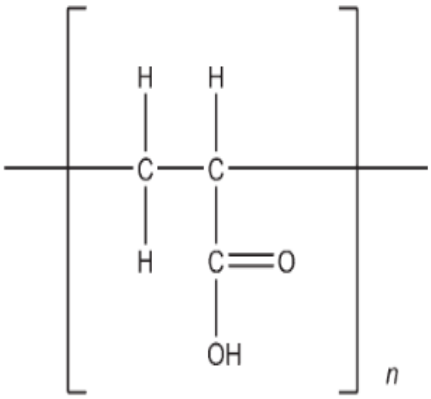
Application

- Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulation.
- It is used as a stabilizing agent for emulsions (0.25–3.0% w/v).
- Polyvinyl alcohol is also usedas a viscosity-increasing agent for viscous formulations such as ophthalmic products.
- It is used in artificial tears and contact lens solutions for lubrication purposes.
- Used in sustained-release formulations for oral administration,and in transdermal patches.
- Polyvinyl alcohol may be made into microspheres when mixed with a glutaraldehyde solution.

Used in Emulsions -0.5 % concentration,
Ophthalmic formulations - 0.25–3.00% concentration ,
Topical lotions- 2.5% concentration.

CARBOMER (CARBOPOL 934)[79]

Structure



Synonyms

Acrypol; Acritamer; acrylic acid polymer; carbomera; Carbopol;carboxypolymethylene; polyacrylic acid; carboxyvinyl polymer;Pemulen; TegoCarbomer.

Empirical formula

($C_3H_4O_2)_n$

Molecular weight

The molecular weight of carbomer is theoretically estimated at $7 – 10^5$to $4 -10^9$

Functional category

Bioadhesive material; controlled-release agent; emulsifying agent;emulsion stabilizer; rheology modifier; stabilizing agent; suspending agent; tablet binder.

Description

Carbomers are white-colored, ‘fluffy’, acidic, hygroscopic powderswith a characteristic slight odor.

Properties

Melting Point:Decomposition occurs within 30 minutes at 260˚C.

Safety

- Carbomers are used extensively in non parenteral products, particularly topical liquid and semisolid preparations.
- Grades polymerized in ethyl acetate may also be used in oral formulations.
- There is no evidence of systemic absorption of carbomer polymers following oral administration.
- Acute oral toxicity studies in animals indicate that carbomer 934P has a loworal toxicity, with doses up to 8 g/kg being administered to dogswithout fatalities occurring.
- Carbomers are generally regarded as essentially nontoxic and nonirritant materials,there is no evidence in humans of hypersensitivity reactions to carbomers used topically.

Stability andstorage conditions

- Carbomers are stable, hygroscopic materials that may be heated attemperatures below 104˚C for up to 2 hours without affecting theirthickening efficiency.
- Carbomer powder should be stored in an airtight, corrosionresistantcontainer and protected from moisture. The use of glass, plastic, or resin-linedcontainers is recommended for the storage of formulations containing carbomer.

Regulatory status

- Included in the FDA Inactive Ingredients Database (oral suspensions, tablets; ophthalmic, rectal, topical, transdermal preparations;vaginal suppositories).
- Included in non parenteral medicines licensed in Europe. Included in the Canadian List of Acceptable Nonmedicinal Ingredients.

Application

- In tablet formulations, carbomers are used as controlled release agents and/or as binders.
- Higher viscosity does not result in slower drug release with carbomers.

Uesd as Emulsifying agent -0.1–0.5 %
Gelling agent -0.5–2.0 %
Suspending agent - 0.5–1.0 %
Tablet binder -0.75–3.0 %
Controlled-release agent- 5.0–30.0 %

The List of excipients used and their Manufacturers are in the Table : 5.

Table-5 : List of Materials Used

S.No.	DRUG/EXCIPIENTS	MANUFACTURER
1.	Nystatin	Caplin point Laboratories Pvt Ltd.
2.	Ethyl cellulose	Caplin point Laboratories Pvt Ltd.
3.	Polymethyl methacrylate	Lab chemicals.
4.	Polyvinyl alcohol	Lab chemicals.
5.	Dichloromethane	Chen chems labs.
6.	Dimethyl sulfoxide	Chen chems labs.
7.	Potassium dihydrogen ortho phosphate	Supra chemicals, Chennai.
8.	Disodium hydrogen ortho phosphate	Supra chemicals, Chennai.
9.	Methanol	Sisco research laboratories Pvt Ltd.
10.	Dialysis Bag (LA653)	Hi media Lab, Mumbai.
11.	Carbopol 934	Sai Mirra Innopharm Pvt. Ltd.
12.	Glycerin	Microlabs Pvt. Ltd.
13.	Triethanolamine	Microlabs Pvt. Ltd.

The List of Equipments used in the study and their Manufacturer are in Table- 6

Table-6 : List of Equipments Used.

S.No	EQUIPMENTS	MANUFACTURER
1.	High Speed Homogenizer	Remi,Vasai.
2.	Ultra sonicator	Lark.
3.	Electronic balance	McDalal,, Chennai.
4.	Magnetic Stirrer with Hot plate	Remi,Vasai.
5.	UV-Visible Spectrophotometer	Shimadzu,Japan.
6.	High speed cooling centrifuge	Remi,Vasai.
7.	Binocular microscope	Olimpus.
8.	Malvern Zeta Sizer	Malvern,Germany.
9.	Scanning electron microscope	Hitachi,Japan.
10.	FT-IR Spectrophotometer	Nicolet,India.
11.	pH meter	Symchrony.India.
12.	Freeze dryer	McDalal,, Chennai.
13.	Hot air oven	McDalal,, Chennai.
14.	Stability Chamber	Remi CHM 6+.

METHODOLOGY

COMPATIBILITY STUDIES FOR DRUG AND EXCIPIENTS[28]

For the development of formulation dosage form, preformulation studies were carried out to confirm no interaction between the drug and excipients. It gives information needed for selection of excipients with the drug for the formulation of Nanosponge. Physical compatibility of the drug with excipients were done. The possibility of drug excipients (Ethyl cellulose and Polymethyl methacrylate) interaction were investigated by FT-IR Spectrum study.

Physical Compatibility

Physical compatibility of the drug and excipients were carried at Room temperature and at 40˚C±2˚C/75±5%RH(in days) with the physical admixture of drug and excipients.

Fourier Transforms Infrared (FT-IR)Spectroscopic studies

The spectroscopic studies were carried out to find out the interaction between pure drug, excipients and its physical mixture by KBr pellet technique using FT-IR spectrophotometer. The IR spectrum of the physical mixture is then compared with the spectrum of pure drug(Nystatin)to assess the compatibility of the excipients and drug. The scanning range is 450-4000 $cm^{-1}$and the resolution is 4cm^{-1}.

PREPARATION OF PHOSPHATE BUFFER pH 5.5[69]

Solution I – 13.612 g of Potassium dihydrogen phosphate in 1000 ml of Distilled Water.

Solution II – 35.08 g of Disodium hydrogen phosphate in 1000 ml of Distilled Water.

96.4 ml of Solution I and 3.6 ml of Solution II were mixed together.

STANDARD CURVE OF NYSTATIN[28,29]

100mg of drug was accurately weighed and dissolved in 30 ml methanol and made up to 100ml with phosphate buffer pH 5.5. Calibration curve was prepared in a mixture of phosphate buffer and methanol (7:3) at λ max 305 nm.

FORMULATIONDEVELOPMENT

FORMULATION OF NYSTATIN LOADED NANOSPONGE[30]

Nystatin Nanosponges were prepared by Emulsion solvent evaporation method. Two different polymers were used in the formulation. Ethyl cellulose (EC) and Polymethyl methacrylate (PMMA) were the Polymers used. Polyvinyl alcohol in Distilled water is used as the aqueous phase. The Drug is dissolved in the required solvent (Dimethyl sulphoxide) and the Polymers (1:1, 1:2, 1:3, 1:4, and 1:5) were dissolved in Dichloromethane. The Drug solution was poured into the polymer solution and the mixture was shaken well. Then the Drug polymer mixture was poured into the aqueous phase and the mixer is subjected to homogenization using High speed homogenizer in 1500 rpm for 2 hours at 35˚C.The formed Nanosponges were centrifuged by high speed cooling centrifuge and the residue was freeze dried.

Table-7 : Formulation of Nanosponge

S.No	INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1.	Drug (mg)	100	100	100	100	100	100	100	100	100	100
2.	EC(mg)	100	200	300	400	500	-	-	-	-	-
3.	PMMA (mg)	-	-	-	-	-	100	200	300	400	500
4.	PVA (g)	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3
5.	DCM (ml)	20	20	20	20	20	20	20	20	20	20
6.	Distilled water(ml)	100	100	100	100	100	100	100	100	100	100

EC-Ethyl cellulose, PMMA – Polymethyl methacrylate, DCM – Dichloromethane, PVA-Polyvinyl Alcohol.

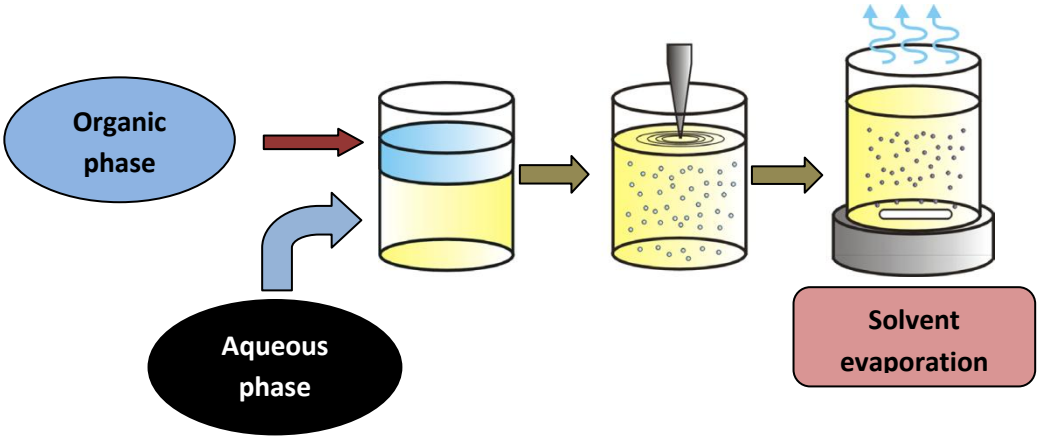


Figure-11. Schematic Representation of Preparation of Nanosponge

CHARACTERIZATION OF NYSTATIN LOADED NANOSPONGE

All the formulated Nystatin loaded Nanosponge were evaluated for its Drug content, entrapment efficiency, particle size distribution, polydispersity index ,*in vitro* drug release and kinetics of drug release.

Determination of drug content[31]

The total drug content of Nanosponge was determined by spectrophotometric analysis.10milligramequivalentof Nystatin loaded Nanosponge taken in a beaker(closed to avoid evaporation) containing (10 ml)of Methanol and stirred for 30 minutes in magnetic stirrer, 3ml of that solution is pipetted out and that volume was made upto10ml by using pH5.5phosphatebuffer to make1µg/ml concentration. The absorbance was measured at 305nm λ_{max} using UV spectrophotometer. From the absorbance drug content was calculated.

The percentage Drug Content is calculated by following formula:

$$\%\text{Drug content} = \frac{\text{Practical Drug Content}}{\text{Theoretical drug content}} \times 100$$

Determination of drug entrapment efficiency[28]

The entrapment efficiency was determined by measuring the concentration of the drug in the supernatant after centrifugation. The unentrapped Nystatin were determined by adding 10 mg Nystatin loaded Nanosponge in 10 ml of methanol and then the dispersion were centrifuged at 9,000rpm for 30 minutes at 4ºC using a cooling centrifuge in order to separate entrapped from the unentrapped drug. The free drug concentration in supernatant layer after centrifugation is determined at λ_{max}(305nm) using UV Spectrophotometer

The percentage entrapment efficiency(%EE) is calculated by following formula:

$$\%EE = \frac{\text{Weight of Initial drug- Weight of free drug}}{\text{Weight of Initial drug}} \times 100.$$

***in- vitro* releasestudies[28]**

The *in vitro* release of Nystatin from Nanosponge was evaluated by the Dialysis Bag diffusion technique. The release studies of Nystatin from Nanosponge were performed in Phosphate buffer of pH5.5 and methanol (70:30) 18mg equivalent Nystatin Nanosponge were suspended in 10 ml of buffer pH 5.5 mixture and placed in the dialysis bag(donor compartment) and sealed at both ends. The dialysis bag were immersed in receptor compartment containing 100ml of buffer mixture, which was stirred at 100 rpm and maintain 32 ± 2˚C. The receptor compartment was covered to prevent evaporation of the diffusion medium. Samples were taken from the receptor compartment and the same amount were replaced with the diffusion medium. Samples are taken upto 24hrs. Nystatin in the samples were measured spectrophotometrically at λ 305nm.

Same procedure was carried out for Pure drug and *in vitro* release were measured spectrophotometrically at λ 305nm.

SELECTION AND EVALUATION OFOPTIMIZEDFORMULATION

The best formulation selection based on the results obtained from particle size, entrapment efficiency, *in-vitro* release studies and kinetics of drug release.

FT-IR spectroscopicstudies

The compatibility study was carried out for optimized formulations(F3 and F7) using FTIR at wavelength range of 4000to400cm^{-1}.Spectrum for optimized formulation were taken and compared.

Morphology ofNanosponge by scanning electronmicroscopy (SEM) technique[32]

The Surface Morphology of the Nanosponge can be measured by SEM. The formulations are poured in a circular aluminum stubs using double adhesive tape, and coated with gold in HUS– 5GB vaccum evaporator and observed in Hitachi S– 3000N SEM at an acceleration voltage of 10 Kv and a magnification of5000X.

Particle size distribution[33]:

Particlesize (z-averagediameter), and polydispersity index (as a measure of the width of the particle size distribution)of Nystatin loaded Nanosponge dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy(PCS) using a Malvern Zeta sizer 3000NanoS(Malvern instruments, UK) at 25°C.

Prior to measurements all samples were diluted using ultra–purified water to yield a suitable scattering intensity. The diluted nanosponge dispersion was poured into disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement.

Kinetics of drug release[78].

To analyze the drug release mechanism, *in-vitro* release are fitted into a

- Zero-order
- First order
- Higuchi,
- Hixon-Crowell cube root law,
- Korsmeyer- peppas model.

The **zero order rate** describes the systems where the drug release rate is independent of its concentration. It is ideal for the formulation to have release profile of zero order to achieve pharmacological prolonged action.

$$\mathbf{Q_t = Q_0 + K_0 t}$$

Where, Q_t is the amount of drug dissolved in time t,

Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and

K_0 is the zero order release constant expressed in units of concentration/time.

The **first order rate** describes the release from a system where the release rate is Concentration dependent.

$$\mathbf{\log C = \log C_0 - k_t / 2.303}$$

Where, C is the concentration of the drug at time(t),

C_0 is the initial concentration of the drug and

k is the first-order release rate constant.

Higuchi described the release of drugs from porous, insoluble matrix as a square root of time dependent process based on Fickian diffusion.

$$\mathbf{Q = kt^{1/2}}$$

Where Q is the amount of drug released in time t.

k constant reflecting design variable system (Differential rate constant) and

t Time in hours

Hixson and Crowell recognized that the particles diameter and regular area is proportional to the cube root of its volume.

$$\mathbf{W_0^{1/3} - W_t^{1/3} = \kappa t}$$

Where W_0 is the initial amount of drug in the pharmaceutical dosage form,

$W_t$is the remaining amount of drug in the pharmaceutical dosage form at time t and κ(kappa) is a constant incorporating the surface-volume relation.

Korsmeyer–Peppas describes the fraction of drug release relates exponentially with respect to time.

$$M_t / M_\infty = Kt^n$$

Where M_t / M_∞ is a fraction of drug released at time t,

K is the release rate constant and n is the release exponent.

n is diffusional exponent indicative of the mechanism of the drug release.

The diffusion mechanism is given by the slope (n) value,

Table -8: diffusion mechanism

n value	**Mechanism**
0.45	Fickian diffusion
0.45< n< 0.89	Anamolous or non-Fickian diffusion
0.89	Case-II transport
n> 0.89	Super case -II transport

The models were used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism was not known or more than one type of release was involved, the r^2 and K values were calculated for the linear curve obtained by regression analysis of the above plots.

STABILITY STUDIES

The stability studies of the optimized Nanosponge were performed at different conditions of temperature and the effect on physical characteristic, entrapment efficiency and drug content was noted. The Nanosponge were kept in the airtight container and stored at 40±2˚C and in Relative humidity 75±5% for 45 days. The samples were analyzed for the above parameter in 15days,30 days and 45days.

The samples were withdrawn on 15 days, 30days and 45days and checked for changes in Physical appearance and drug content as per ICH QIA (R_2) guidelines.

Table-9 : ICH guidelines ICH QIA (R_2)[38]

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term*	25˚C/60% RH ± 5% or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

*It is up to the applicant to decide whether long term stability studies are performed at 25 ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH.

**If 30°C ± 2°C/65% RH ± 5% RH is the long-term condition, there is no intermediate condition.

FORMULATION OF NYSTATIN LOADED NANOSPONGE GEL[74,75]

The formulation of Nanosponge prepared using the optimized ratio of Polymer containing Nystatin equivalent to18mg was incorporated into the gel base composed of Carbopol 934 (1%), Glycerol (15%), Triethanolamine (q.s.) and distilled water up to 1g.

Table-10: Preparation of Gel

S.No.	INGREDIENTS	GEL BASE
1.	Carbapol 934	1 %
2.	Glycerol	15 %
3.	Triethanolamine	Quantity Sufficient
4.	Distilled water	Upto 15g

EVALUATION OF NANOSPONGE GEL

Physical Appearance

The prepared gel was examined for clarity, color, homogeneity and the presence of foreign particles.

pH

2.5g of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of the dispersion was measured by using a digital pH meter.

Rheological study

Viscosity measurement:

Viscosity was determined by Brookfield viscometer. In the present study, spindle no. S64 with an optimum speed of 0.6 rpm was used to measure the viscosity of the preparation.

Content Uniformity:

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 9mg of the drug in a beaker containing 10 ml of methanol, stir the solution for 30 minutes and centrifuged in High speed cooling centrifuge and 3ml of the solution is made upto 10 ml with phosphate buffer pH 5.5. The samples were analyzed spectrophotometrically at λmax 305 nm against blank using UV- Visible spectrophotometer.

***In-vitro*Drug Diffusion study**

In- vitro drug diffusion study was studied using dialysis bag. The Nanosponge gel equivalent to 18mg of the drug was placed in a Dialysis Bag having 8 cm length and 3 cm breath, both the sides are tied with thread. This acted as the donor compartment. Then the bag was placed in a beaker containing 100 ml phosphate buffered methanol pH 5.5, which acted as receptor compartment. The temperature of the receptor medium was maintained at 37°±2°C and the medium was stirred at a speed of 100 rpm using a magnetic stirrer.

5 ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer PB mixture pH5.5. The sink condition

was maintained throughout the experiment. The collected samples were analyzed Spectrophotometrically at 305 nm using UV- Visible spectrophotometer.

MICROBIOLOGICAL STUDY [49]

Antifungal activity for the prepared Nanosponge gel were carried out. Candida albicans was grown in Malt Extract Agar,(MAE) with medium composition: malt extract 17 g/L and agar 20g/L.

The assay was done using the agar well diffusion method. Sterile agar plates were prepared by pouring the sterilized media in sterile Petri dishes under aseptic conditions.1 mm of the test organism was spread on agar plates. Using a sterile tube with a diameter of 6mm, the wells were made according to the number of samples. The wells were inoculated with 80µL of sample. The antimicrobial activity was interpreted based on the size of inhibition zone (IZ) diameter, which was measured in mm from observation of clear zones surrounding the wells.

PRE-FORMULATION STUDIES

The optimization of a formulation can be done only after a thorough investigation of its physic chemical properties of the drug and excipients. The drug and the polymer must be compatible for a successful formulation.

Physical Compatability

Table-11:Physical compatability

S.No.	Description and condition							
	Drug (D) and excipients	Initial	Room temperature (in days)			40˚C±2˚C/75±5% RH(in days)		
			10	20	30	10	20	30
1.	Drug	NC	NC	NC	NC	NC	NC	NC
2.	D+EC	NC	NC	NC	NC	NC	NC	NC
3.	D+PMMA	NC	NC	NC	NC	NC	NC	NC
4.	D+PVA	NC	NC	NC	NC	NC	NC	NC

NC – No Change.

Inference

The drug and the excipients of the formulation are physically compatible with each other. They were evaluated for 10, 20 and 30 days at room temperature and at 40˚C±2˚C/75±5% Relative Humidity.

Chemical compatability

FT-IR spectroscopy gives the possible information about the interaction between the drug and Polymer.

Drug-Polymer compatibility studies

FT-IR Spectroscopicstudies

The compatibility between drug and Polymer was confirmed using FT-IR Spectroscopy. Infrared spectroscopic analysis for drug (Nystatin),Polymer, Drug-Polymer admixture was carried out.

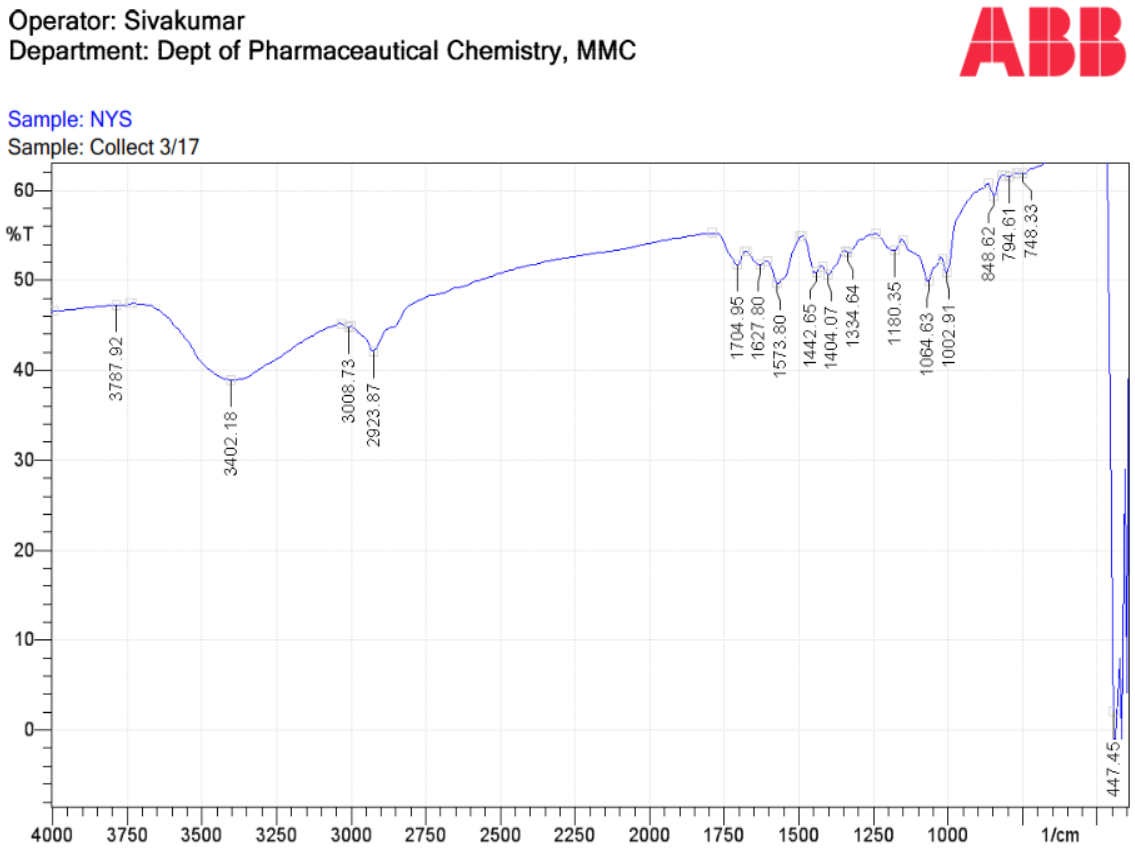


Figure-12 :FT-IR Spectrum of Nystatin.

Table-12 :FT-IR Data of Nystatin.

S.No.	Peak absorbed at Wave number cm^{-1}	Characteristics
1.	3787.90	OH- Stretching
2.	3402.18	NH -Stretching
3.	3008.73	Aromatic CH- Stretching
4.	2923.87	Aliphatic CH- Stretching
5.	1627.80	Carboxylic C=O - Stretching

Inference

The peak shows the presence of NH,OH, aromatic CH, aliphatic CH and Carboxylic C=O groups in the Nystatin sample.

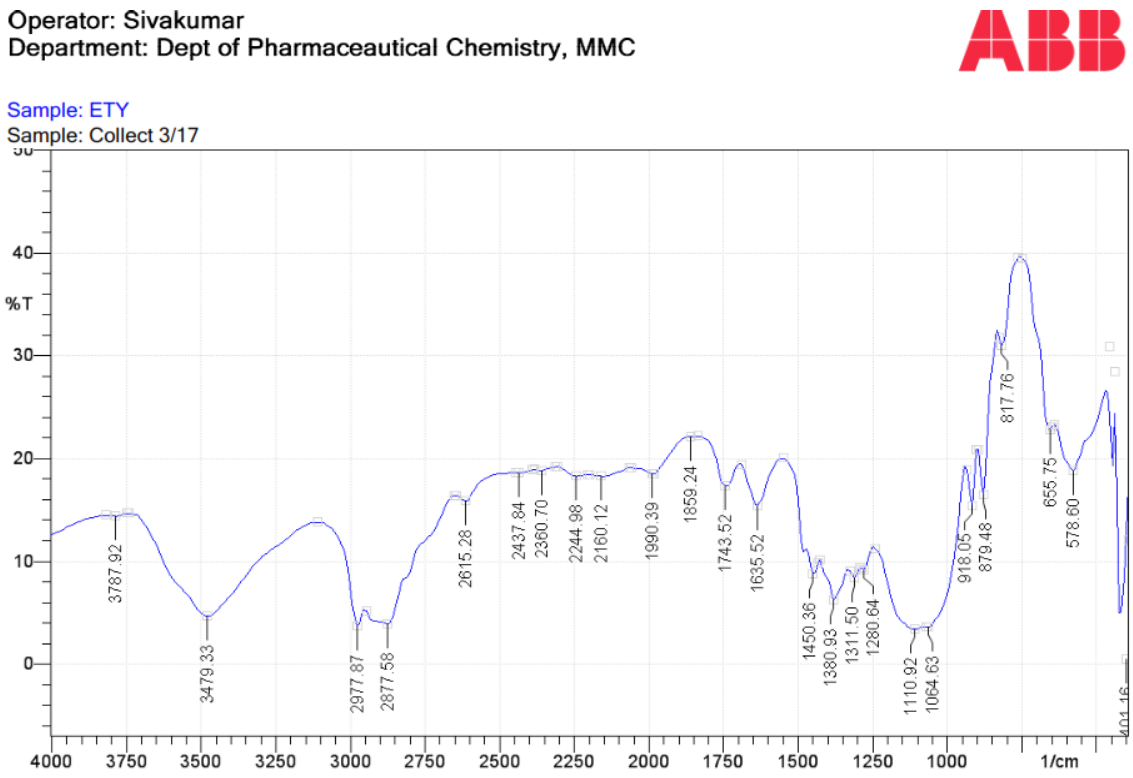


Figure-13 :FT-IR Spectrum of Ethyl cellulose.

Table-13::FT-IR Data of Ethyl cellulose.

S.No.	Peak absorbed at Wave number cm^{-1}	Characteristics
1.	2977.87	Aliphatic CH - Stretching
2.	1635.52	CH(methyl)-Stretching

Inference

The peak shoes that the Ethyl cellulose has Characteristics Aliphatic CH and Methyl CH – streching.

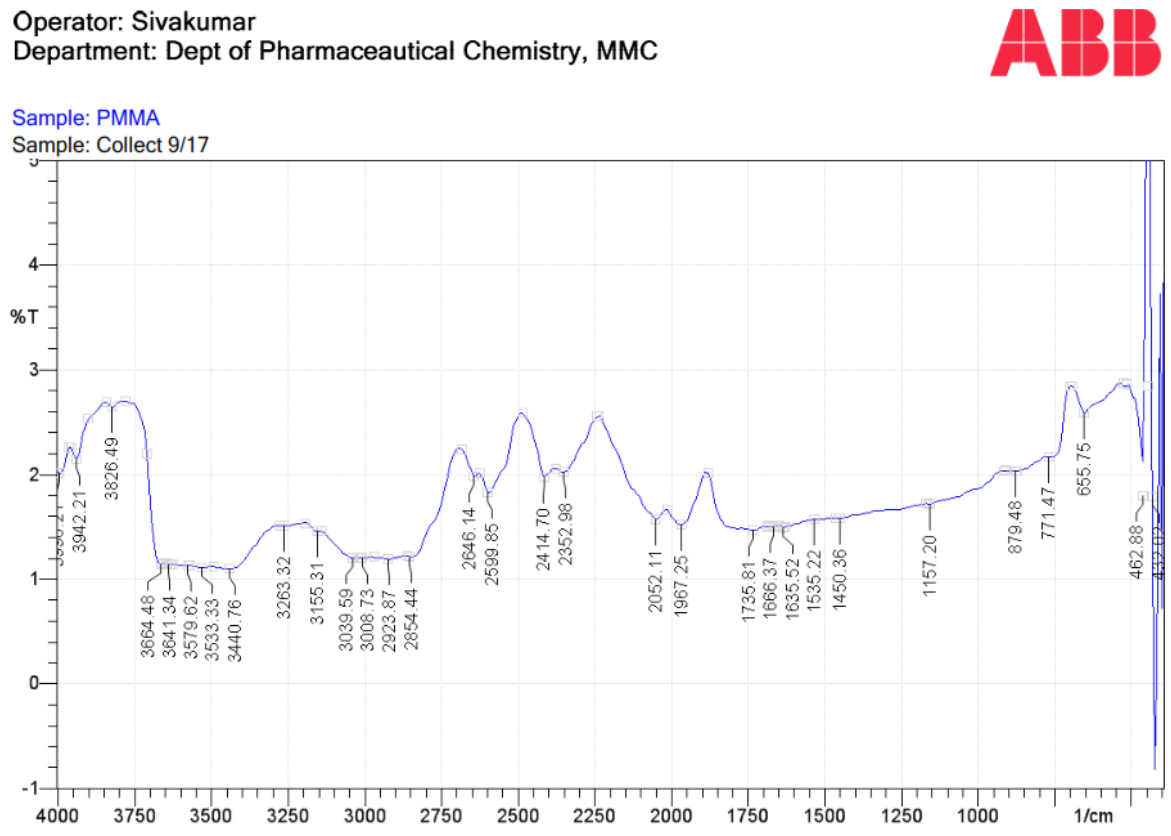


Figure-14 :FT-IR Spectrum of Polymethyl methacrylate.

Table-14 :FT-IR Data of Polymethyl methacrylate.

S.No.	Peak absorbed at Wave number cm^{-1}	Characteristics
1.	1735.81	C=O - Stretching
2.	2923.87	Aliphatic CH -Stretching
3.	1535.22	CH (methyl)- Stretching

Inference

The peak shows that the Characteristics C=O, Aliphatic CH and Methyl CH – stretching.

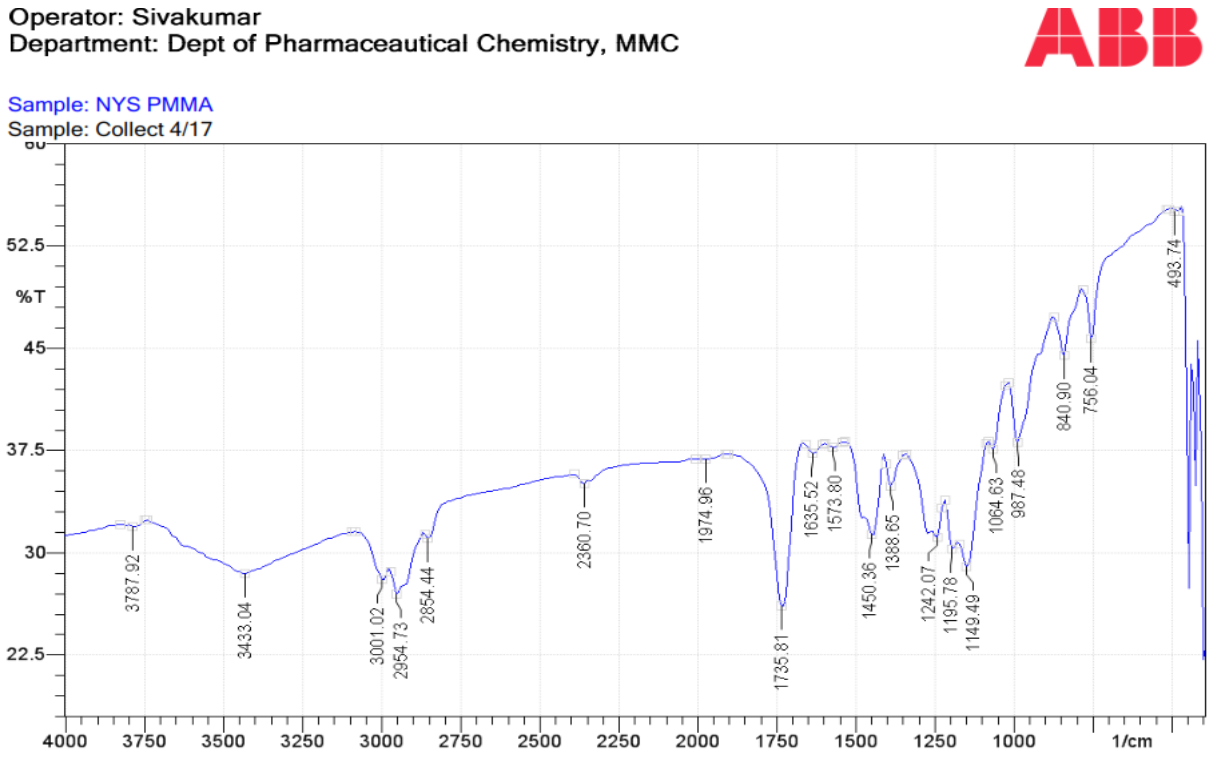


Figure-15 :FT-IR Spectrum of Nystatin with PMMA.

Table-15 :FT-IR Data of Nystatin with PMMA

S.No.	Peak absorbed at Wave number cm^{-1}	Characteristics
1.	3787.92	OH -Stretching
2	3433.04	NH- Stretching
3.	3001.02	Aromatic CH- Stretching
4.	2954.73	Aliphatic CH- Stretching
5.	2854.44	Carboxylic OH - Stretching
6.	1735.81	C=O- Stretching

Inference

The peak observed in the FTIR Spectrum of Nystatin pure drug with Polymer(PMMA) showed no shift and no disappearance of characteristic peaks of pure drug suggesting no interaction between the drug and Polymer.

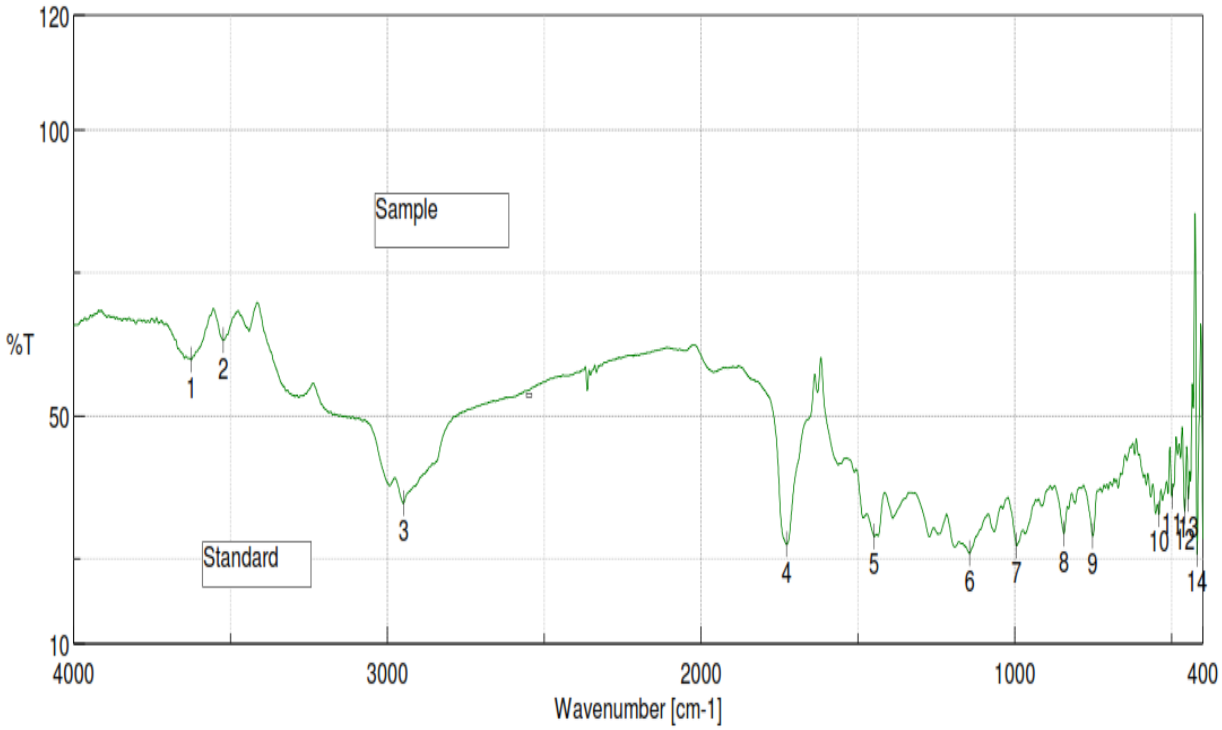


Figure-16 :FT-IR Spectrum of Nystatin with Ethylcellulose

Table-16 :FT-IR Data of Nystatin with Ethylcellulose.

S.No.	Peak absorbed at Wave number cm^{-1}	Characteristics
1.	3787	OH -Stretching
2	3400	NH- Stretching
3.	2954	Aliphatic CH- Stretching
4.	1700	Carboxylic OH - Stretching

Inference

The peak observed in the FTIR Spectrum of Nystatin pure drug with Polymer (Ethyl cellulose) showed no shift and no disappearance of characteristic peaks of pure drug suggesting no interaction between the drug and Polymer[29].

STANDARD CURVE OFNYSTATIN

The UV spectrometric method was used to analyse Nystatin. The absorbance of the drug in phosphate buffered methanol pH 5.5. (70:30) was measured at a wavelength of 305 nm. The results are given in the Table and Figure

Table-17 :Data for calibrationcurve

S.No	Concentration (mcg/ml)	Absorbanceat305 nm
1	0	0
2	2	0.148 ± 0.010
3	4	0.293 ± 0.0086
4	6	0.445 ± 0.026
5	8	0.586 ± 0.0061
6	10	0.756 ± 0.0214

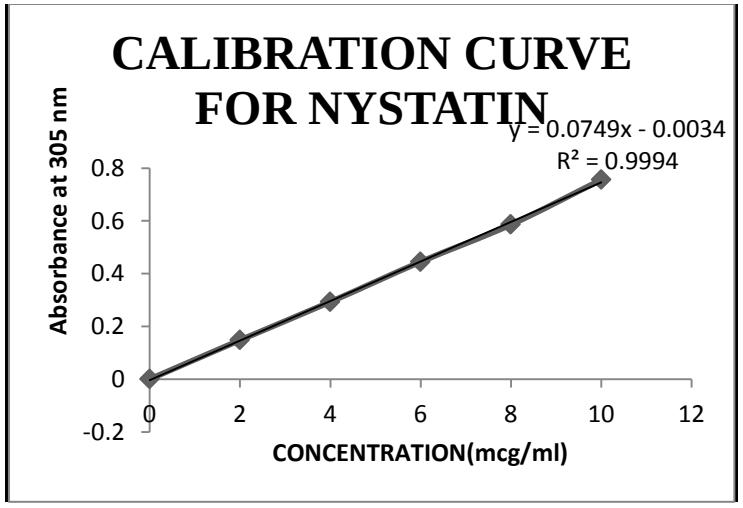


Figure-17 : Calibration curve for Nystatin.

Inference

It was found that the solutions of Nystatin in Phosphate buffered methanol pH5.5 show linearity (R^2=0.999) in absorbance at concentrations of 2-10 mcg/ml and obey Beer Lambert's law [77].

FORMULATION OFNYSTATIN NANOSPONGE

Nystatin Nanosponge were prepared by homogenization technique followed by centrifugation.

Figure-18 : Formulation of Nanosponges

CHARACTERIZATION OF NANOSPONGE

Drug content and entrapment Efficiency

Table-18 : Drug content and Entrapment Efficiency of NystatinNanosponge

FORMULATION CODE	%DRUG CONTENT (%w/v)	%ENTRAPMENT EFFICIENCY (% w/v)
F1	94.52±0.109	98.79
F2	91.31±0.633	98.14
F3	**90.26±0.266**	**98.94**
F4	92.54±1.173	98.86
F5	82.90±1.239	97.85
F6	90.36±0.366	96.57
F7	**95.714±0.231**	**99.21**
F8	85.44±0.555	85.44
F9	88.95±0.524	88.95
F10	86.70±0.708	86.70

DRUG CONTENT

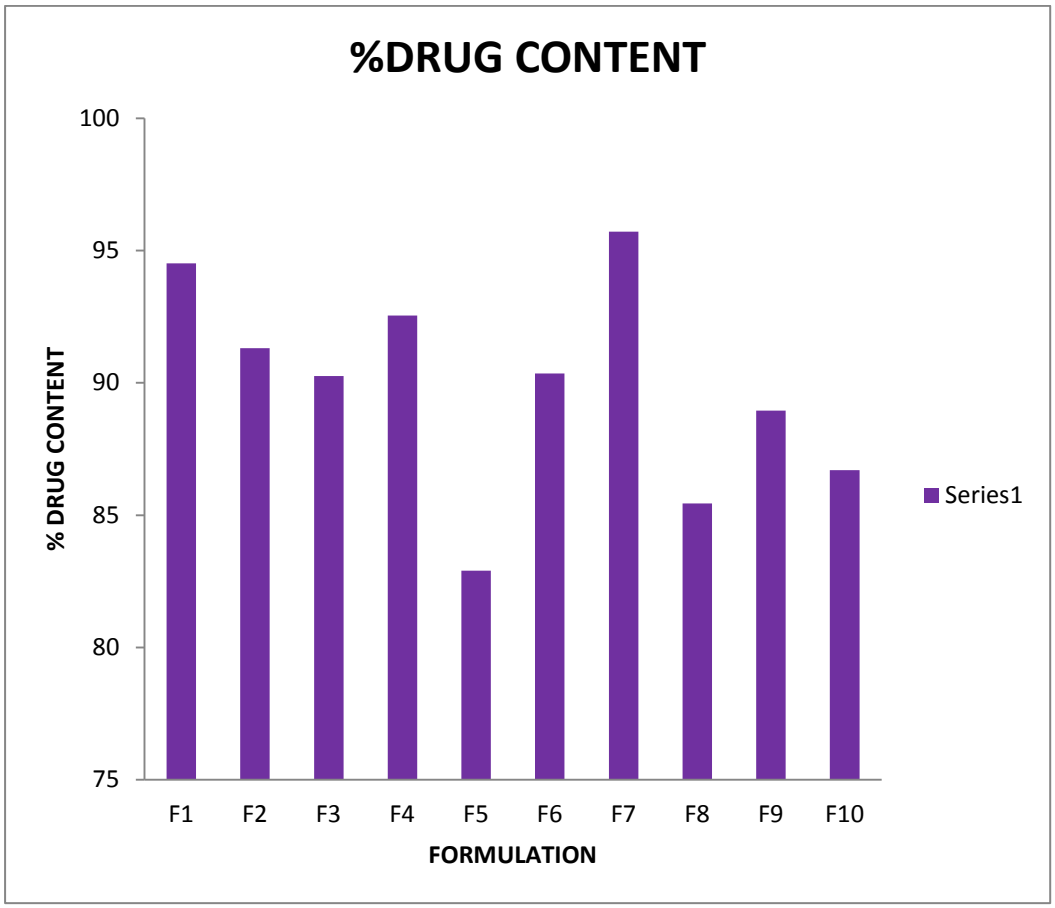


Figure-19 : % Drug content of NystatinNanosponge

Inference

The Drug content of the formulations was observed to be between 82.90 to 95.71.

ENTRAPMENTEFFICIENCY

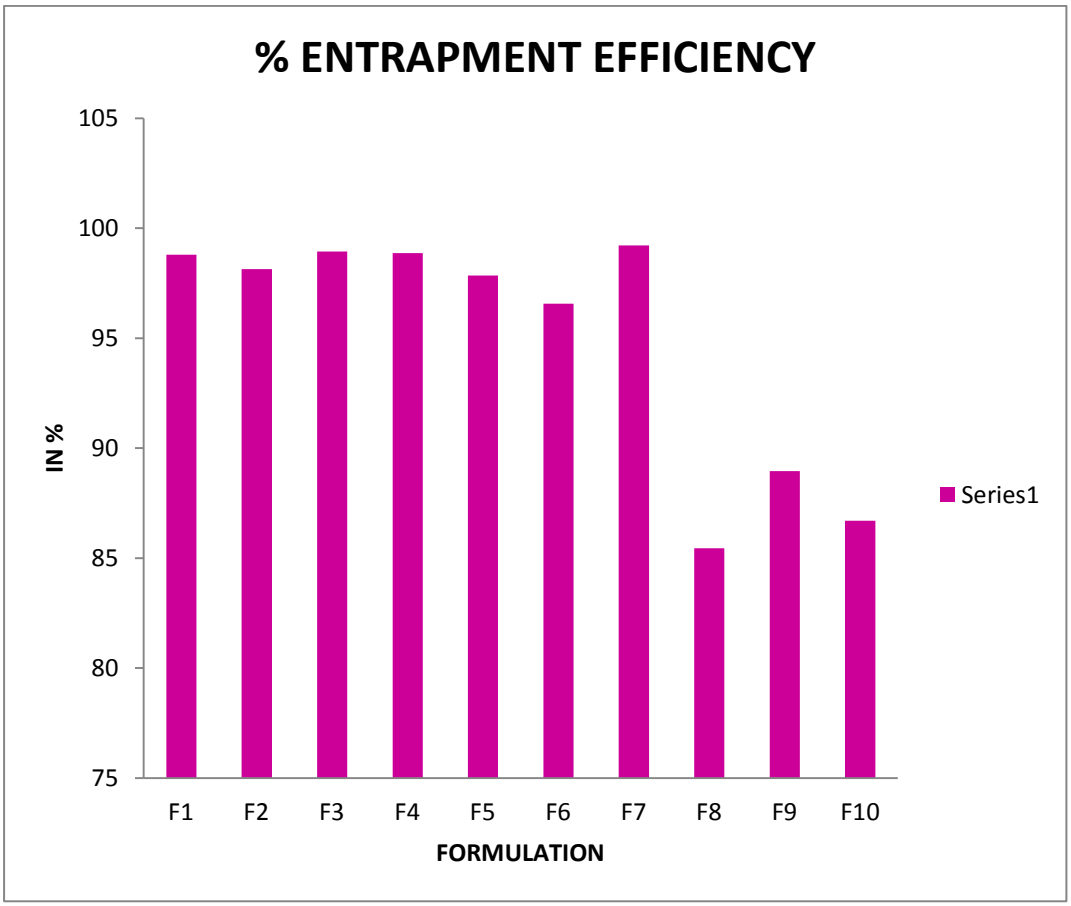


Figure-20 :% Entrapment Efficiency of NystatinNanosponge

Inference

The entrapment efficiency was found to be 97.85 to 99.21. The highest entrapment efficiency was observed with 99.21 and 98.94 for the formulations F3 and F7.

SURFACE MORPHOLOGY OF NANOSPONGE

Surface morphology of Nanosponge was measured by Scanning Electron Microscopy (SEM).

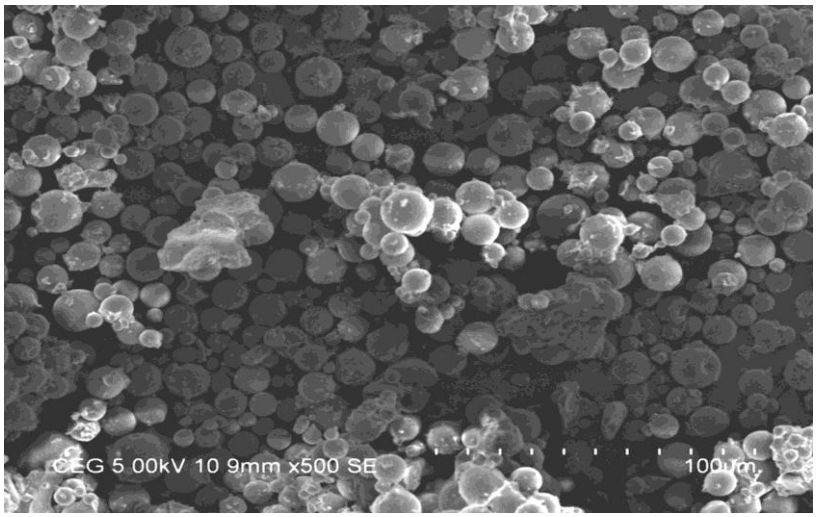


Figure-21 : SEM image of Optimized Formulation (F3)

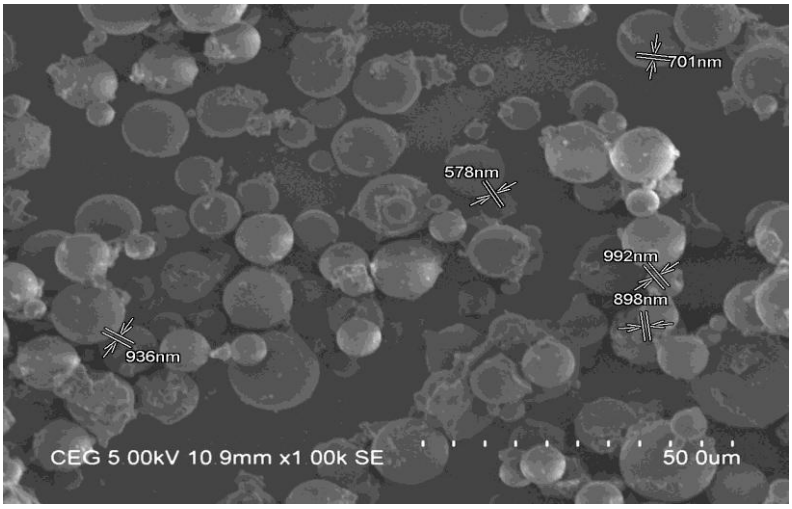


Figure-22 : SEM image of optimized Formulation (F7)

Inference

SEM picture shows the formation of Spherical Nanoparticles.

PARTICLE SIZE DISTRIBUTION BY MALVERN ZETA SIZER:

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	ET 3 1		
SOP Name:	SAMPLE 1-PMMA.sop		
General Notes:			
File Name:	MMC.dts	**Dispersant Name:**	Water
Record Number:	9	**Dispersant RI:**	1.330
Material RI:	1.40	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	25 February 2016 15:36:40

System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	211.4	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	8

Results

			Size (d.nm):	**% Intensity:**	**St Dev (d.n...**
Z-Average (d.nm):	3305	**Peak 1:**	231.1	100.0	19.47
PdI:	1.000	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.916	**Peak 3:**	0.000	0.0	0.000

Result quality : Refer to quality report

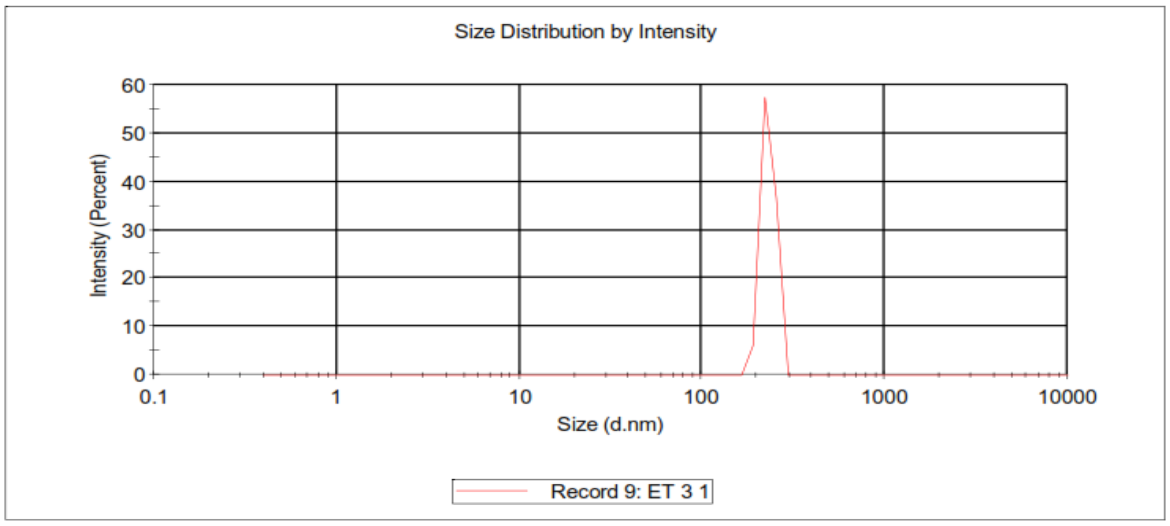


Figure-23 :Size distribution of optimized formulation F3

Inference: The average particle size of Nystatin loaded Nanosponge is 231.1 nm and the poly dispersity index was found to be 1.000

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	PMMA 2 2		
SOP Name:	SAMPLE 1-PMMA.sop		
General Notes:			
File Name:	MMC.dts	**Dispersant Name:**	Water
Record Number:	8	**Dispersant RI:**	1.330
Material RI:	1.40	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	25 February 2016 15:10:46

System

Temperature (°C):	25.0	**Duration Used (s):**	90
Count Rate (kcps):	76.9	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	9

Results

			Size (d.nm):	**% Intensity:**	**St Dev (d.n...**
Z-Average (d.nm):	3747	**Peak 1:**	370.3	100.0	27.00
PdI:	1.000	**Peak 2:**	0.000	0.0	0.000
Intercept:	1.01	**Peak 3:**	0.000	0.0	0.000

Result quality : Refer to quality report

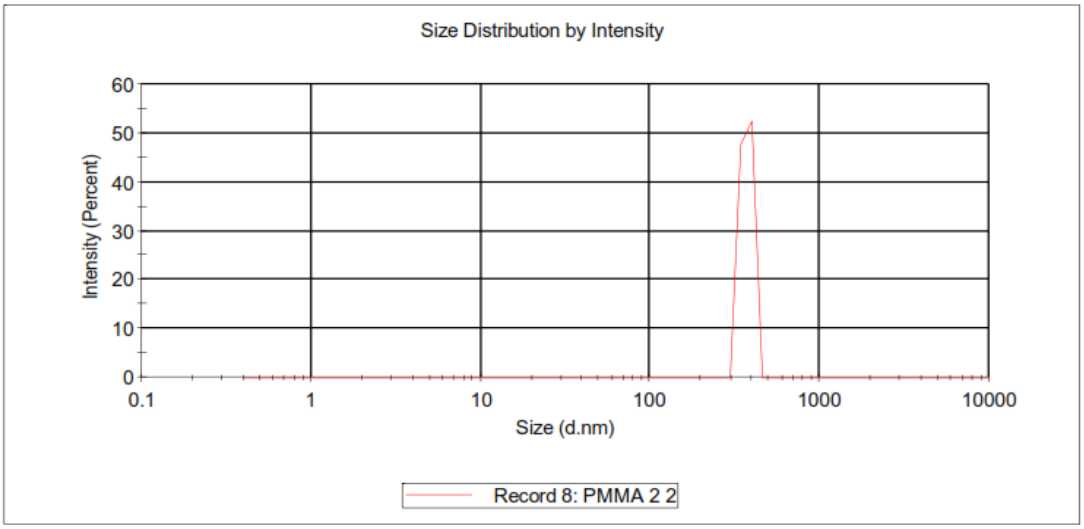


Figure-24: Size distribution of optimized formulation F7

Inference

The average particle size of NystatinNanosponge F7 was 370.3nm and the polydispersity index was found to be 1.000

Table-19 : *in-vitro* drug release of pure drug and NystatinNanosponges (EC)

Time in Hours	PURE DRUG	F1	F2	F3	F4	F5
0	0	0	0	**0**	0	0
1	0.61	1.7	1.74	**2.84**	2.07	1.03
2	0.66	3.49	2.54	**5.06**	3.57	1.8
3	0.84	4.45	3.61	**8.41**	5.29	2.51
4	1	5.68	4.87	**10.69**	6.32	3.83
5	1.17	6.82	4.97	**13.06**	7.71	4.31
6	1.31	7.71	5.71	**14.55**	8.51	4.96
7	1.58	9.28	6.95	**16.75**	10.04	5.79
8	1.74	11.12	8.47	**18.6**	10.66	6.39
9	1.8	12.38	9.07	**19.71**	11.09	7.37
10	1.91	14.03	9.8	**22.51**	11.61	8.73
11	2	16.84	10.79	**24.44**	12.81	9.82
23	2.54	17.97	17.52	**31.92**	17.8	13.75
24	2.63	20.11	23.3	**36.28**	20.46	16.68

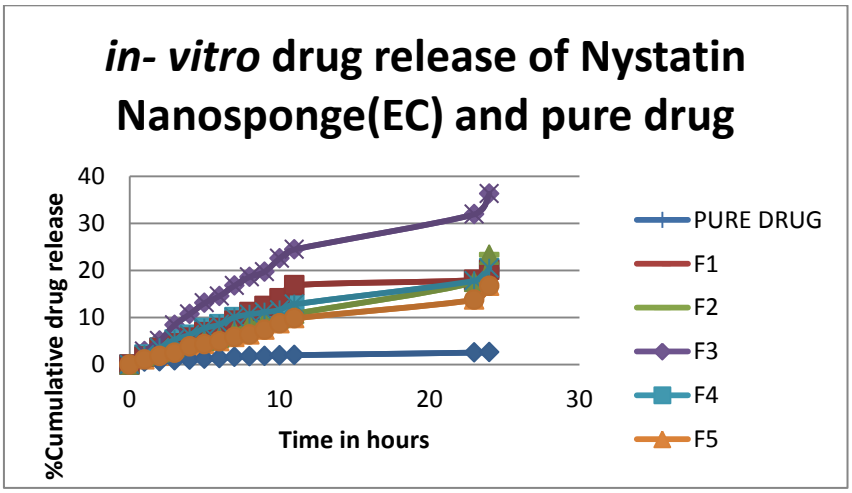


Figure-25: *in- vitro* drug release of Nystatin Nanosponge(EC) and pure drug

Table-20: ***in-vitro*** **drug release of pure drug and Nystatin Nanosponge (PMMA)**

Time in Hours	PURE DRUG	F6	F7	F8	F9	F10
0	0	0	**0**	0	0	0
1	0.61	1.23	**4.14**	1.27	1.63	1.69
2	0.66	2.47	**7.3**	2	2.94	3.34
3	0.84	3.8	**10.16**	2.81	4.06	4.97
4	1	5.75	**14.13**	3.63	5.24	6.83
5	1.17	6.65	**15.94**	4.76	6.47	8.08
6	1.31	7.72	**18.69**	5.25	7.67	9.46
7	1.58	9.85	**20.72**	6.15	8.65	11.5
8	1.74	10.38	**232**	7.16	9.35	12.62
9	1.8	12.23	**27.06**	7.49	10.53	14.73
10	1.91	12.96	**29.16**	8.54	11.49	15.8
11	2	13.79	**35.58**	9.05	12.47	21.85
23	2.54	20.02	**41.45**	14.5	17.61	23.46
24	2.63	22.71	**45.66**	15.35	19.11	25.26

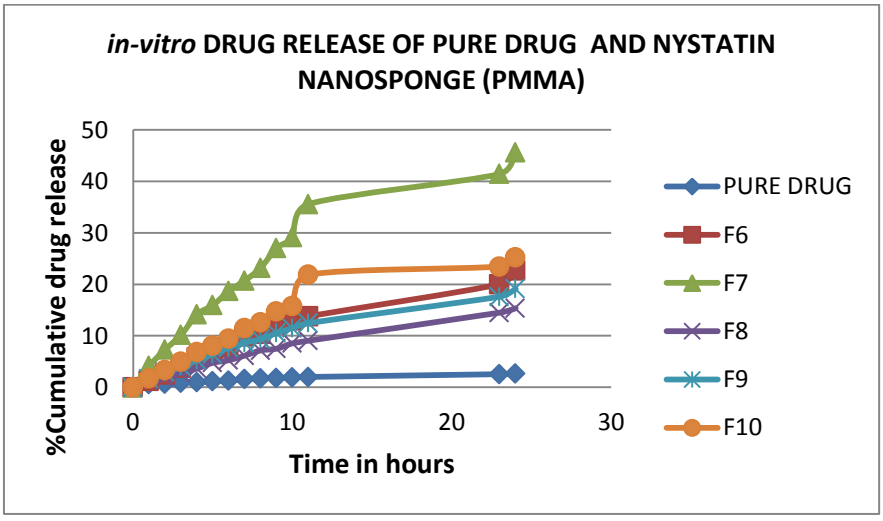


Figure-26 : ***in -vitro*** **drug release of Nystatin Nanosponge (PMMA) with pure drug.**

Inference

The *in-vitro* release of the formulations was observed to be between15.53% to 45.66 %The results shown that the increase in polymer concentration increase the drug release and then it is decreased as described by Dr Prathima Srinivas *et al*[30]. From the result it was observed that the formulations F3and F7Showed sustained release of drug.

Table-21 : *in-vitro* drug release of pure drug, marketed sample (Nistatina cream) and Nystatin Nanosponge gel (EC and PMMA) G1 and G2

Time in hours	Pure drug	Marketed cream	Nanosponge Gel G1	Nanosponge Gel G2
0	0	0	**0**	**0**
1	0.61	0.9	**2.73**	**3.18**
2	0.66	1.2	**5.18**	**5.36**
3	0.84	1.39	**7.39**	**7.28**
4	1	1.51	**8.62**	**9.73**
5	1.17	1.64	**9.28**	**10.73**
6	1.31	1.79	**11.03**	**11.37**
7	1.58	1.92	**12.66**	**14.3**
8	1.74	2.11	**14.24**	**16.06**
9	1.8	2.33	**15.05**	**17.28**
10	1.91	2.5	**15.81**	**18.17**
11	2	2.66	**16.59**	**19.1**
23	2.54	2.87	**21.75**	**26.04**
24	2.63	3.01	**23.15**	**28.88**

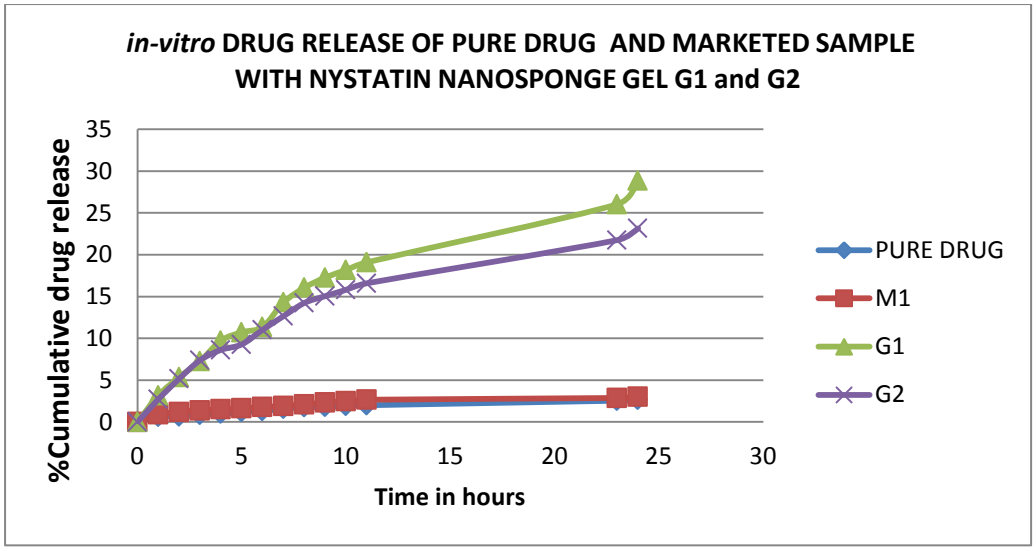


Figure-27: *in-vitro* drug release of Pure drug, Marketed sample (Nistatina cream) and Nystatin Nanosponge gel G1 and G2

Inference

The *in–vitro* release of the Gel formulation of F3 and F7 was compared with marketed sample of Nystatin cream, and the release of Nanosponge gel (G1 and G2) was 23.15% and 28.88% respectively.

The release of gel was increased due to the decreased particle size and increased surface area as described by Rawia M. Khalil *et al*[28].

Figure-28 : Diffusion Study By Dialysis Bag method

KINETICS OFDRUG RELEASE

The*in-vitro*releasedatawasappliedtovariouskineticmodelstopredictthemechanism ofdrugreleaseoftheNanospongeformulations.

Table -22 : Release kinetics of NystatinNanosponge Optimized formulation F3

Time in Hours	%cum drug release	% drug remaining	Log%cum drug remaining	Square root of time	Log time	Cube root of % drug remaining	Log %cum drug release
0	0	100	2	0	∞	4.641589	∞
1	2.84	97.16	1.98748	1	0	4.597225	0.453318
2	5.06	94.94	1.97744	1.41421	0.30102	4.561941	0.704151
3	8.41	91.59	1.96184	1.73205	0.47712	4.507641	0.924796
4	10.69	89.31	1.95090	2	0.60205	4.469922	1.028978
5	13.06	86.94	1.93921	2.23606	0.69897	4.430028	1.115943
6	14.55	85.45	1.93171	2.44949	0.77815	4.404575	1.162863
7	16.75	83.25	1.92038	2.64575	0.84509	4.366445	1.224015
8	18.6	81.4	1.91064	2.82842	0.90308	4.333859	1.269513
9	19.71	80.29	1.90466	3	0.95424	4.314069	1.294687
10	22.51	77.49	1.88924	3.16227	1	4.263326	1.352375
11	24.44	75.56	1.87829	3.31662	1.04139	4.227633	1.388101
23	31.92	68.08	1.83302	4.79583	1.36172	4.083255	1.504063
24	36.28	63.72	1.80427	4.89897	1.38021	3.994158	1.559667

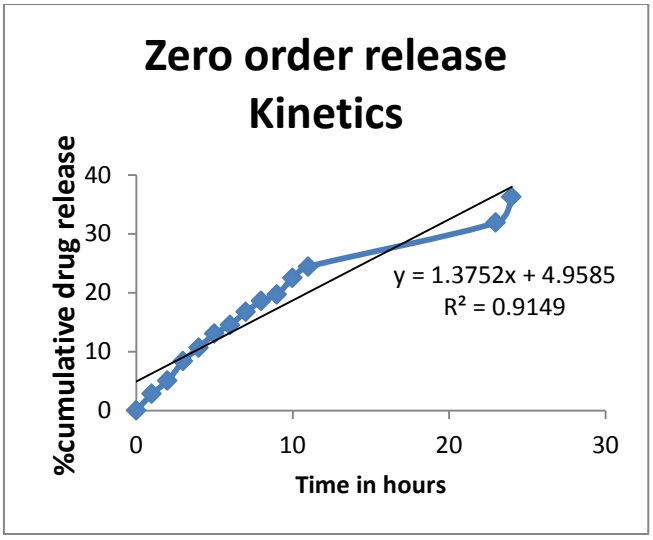


Figure-29: Zero OrderKineticsof F3

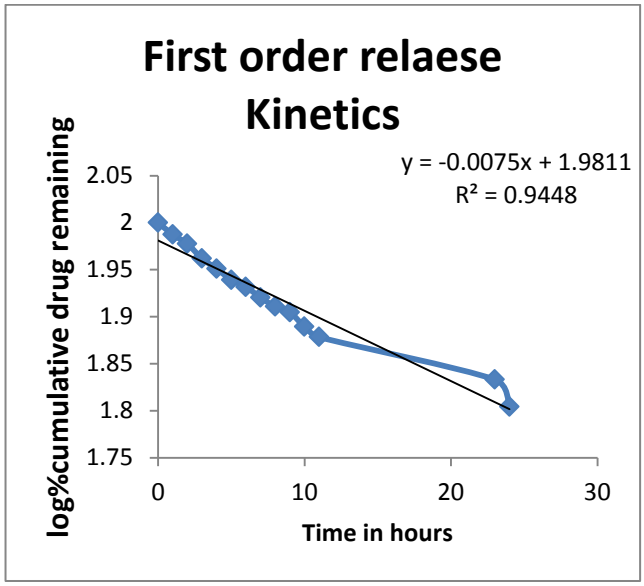


Figure-30 :FirstOrderKinetics of F3

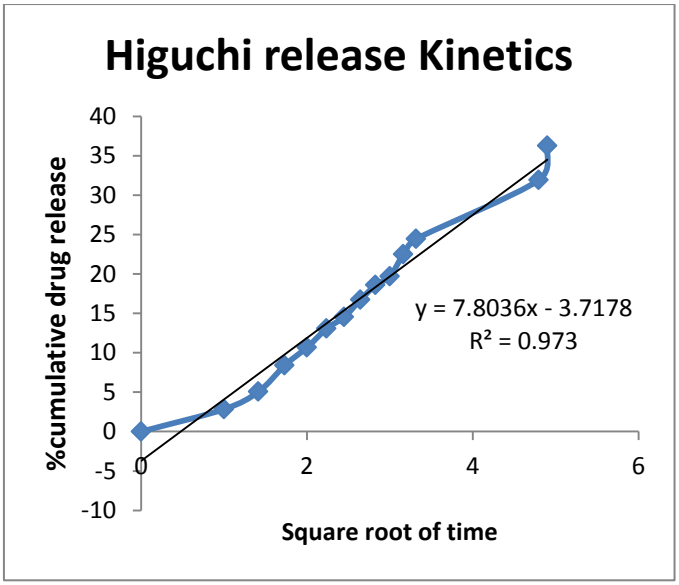


Figure-31: Higuchi ReleaseKinetic of F3

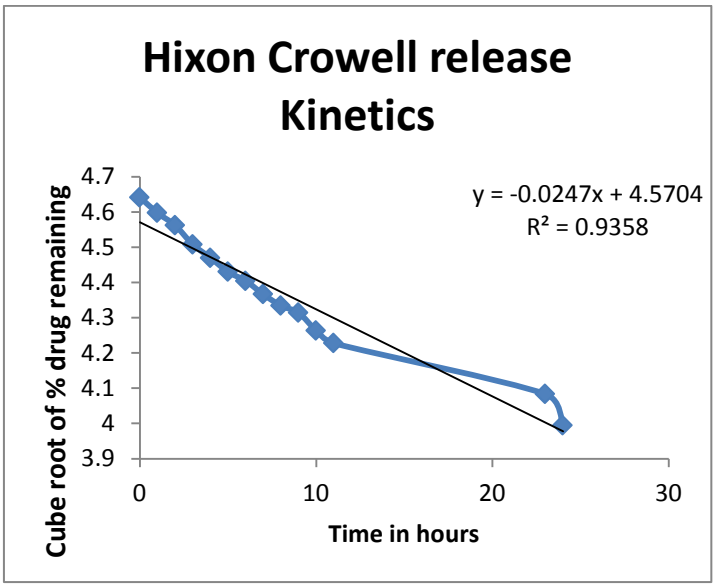


Figure-32:HixonCrowellReleaseKinetics of F3

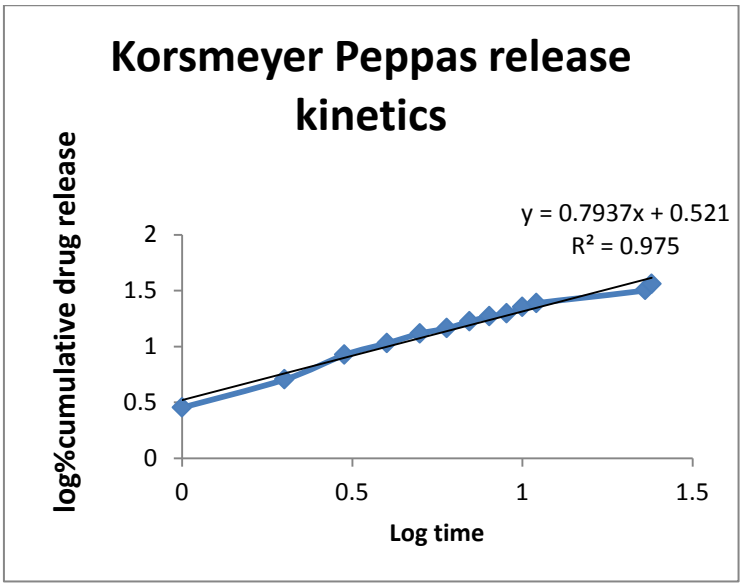


Figure-33:KorsmeyerPeppas Release Kinetics of F3

Table-23 : Release kinetics of F3

S.No.	Release Kinetics	X-axis	Y-axis	Slope	Intercept	R^2	Linear Equation
1.	Zero order equation	Time in hours	Cumulative %drug release	1.375	4.958	0.914	y=1.375x+4.958 R^2=0.914
2.	First order equation	Time in hours	Log% cumulative drug release	-0.007	1.981	**0.944**	y=-0.007x+1.981 R^2 = 0.944
3.	Higuchi release kinetic	Square root of time	Cumulative %drug release	7.803	-3.717	0.973	y=7.803x-3.717 R^2 = 0.973
4.	Hixon Crowell equation	Time in hours	Cube root of % drug release	-0.024	4.570	0.935	y=-0.024x+4.570 R^2 = 0.935
5	Korsmeyer Peppas equation	Log time	Log % cumulative drug release	0.793	0.521	**0.975**	y=0.793x+0.521 R^2 = 0.975

Inference

- Formulation F3 followed first- kinetics and their R^2 value 0.944 indicating the release to be dose dependent.
- The drug release was proportional to the square root of time indicating that Nystatin release from Nanosponge was diffusion controlled.
- The n value for the korsmeyer- peppas for formulation F3 was found to be 0.793. So the formulation F3 non-Fickian type mechanism.

- The Drug release pattern from Formulation F3 follows First order release, Higuchi model and Non-fickian transport.

Table-24 : Release kinetics of Nystatin Nanosponge Optimized formulation F7

Time in Hours	%cum drug release	% drug remaining	Log% cum drug remaining	Square root of time	Log time	Cube root of % drug remaining	Log %cum drug release
0	0	100	2	0	∞	4.641588	∞
1	4.14	95.86	1.981637	1	0	4.57663	0.617
2	7.3	92.7	1.96708	1.414214	0.30103	4.525777	0.863323
3	10.16	89.84	1.95347	1.732051	0.477121	4.478747	1.006894
4	14.13	85.87	1.933841	2	0.60206	4.411779	1.150142
5	15.94	84.06	1.924589	2.236068	0.69897	4.380561	1.202488
6	18.69	81.31	1.910144	2.44949	0.778151	4.332261	1.271609
7	20.72	79.28	1.899164	2.645751	0.845098	4.295903	1.31639
8	23.2	76.8	1.885361	2.828427	0.90309	4.250634	1.365488
9	27.06	72.94	1.862966	3	0.954243	4.178193	1.432328
10	29.16	70.84	1.850279	3.162278	1	4.137704	1.464788
11	35.58	64.42	1.809021	3.316625	1.041393	4.00873	1.551206
23	41.45	58.55	1.767527	4.795832	1.361728	3.883073	1.617525
24	45.66	54.34	1.73512	4.898979	1.380211	3.787679	1.659536

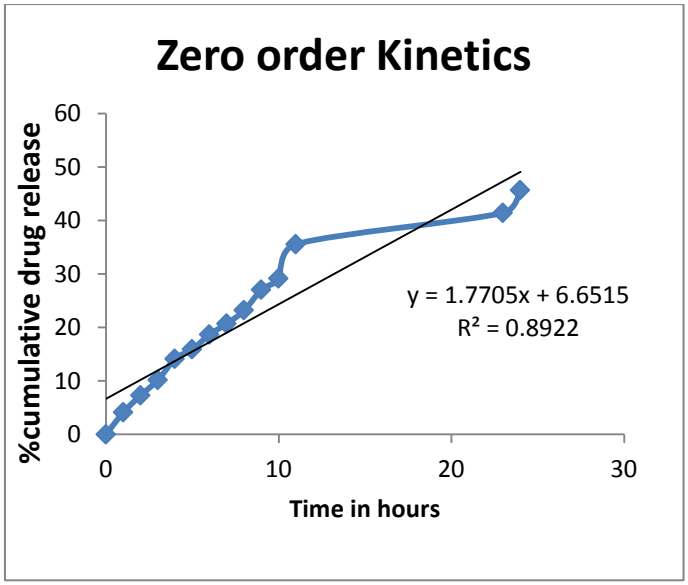


Figure-34: Zero OrderKineticsof F7

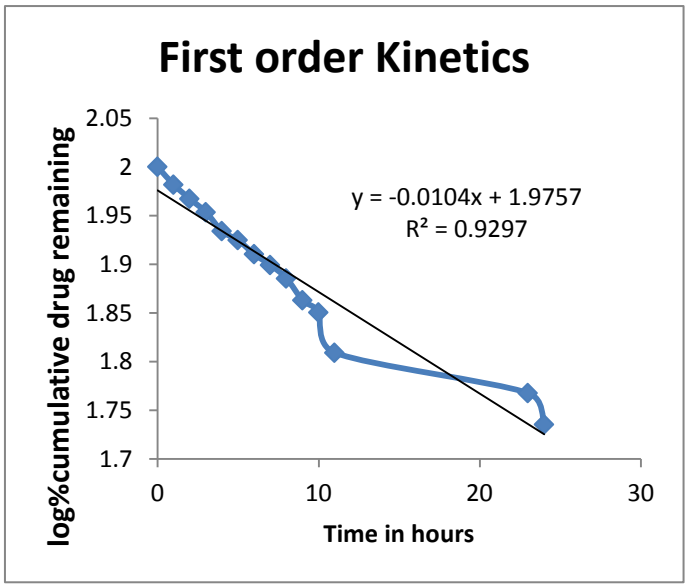


Figure-35: First OrderKineticsof F7

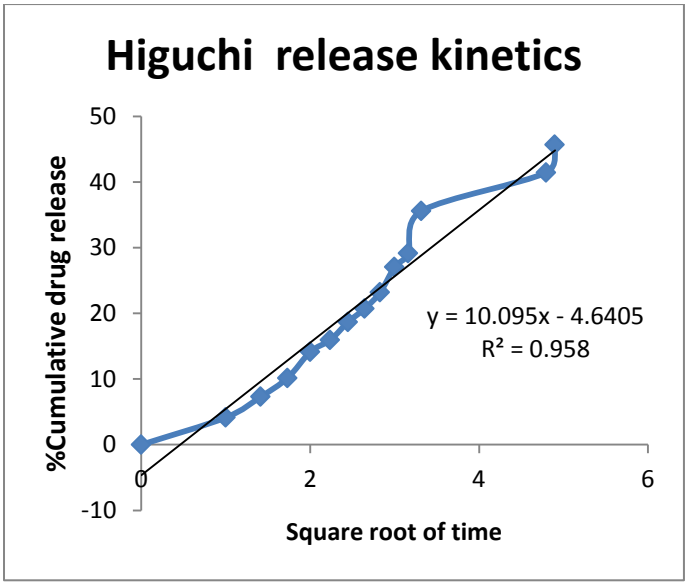


Figure-36:Higuchi Release kinetics of F7

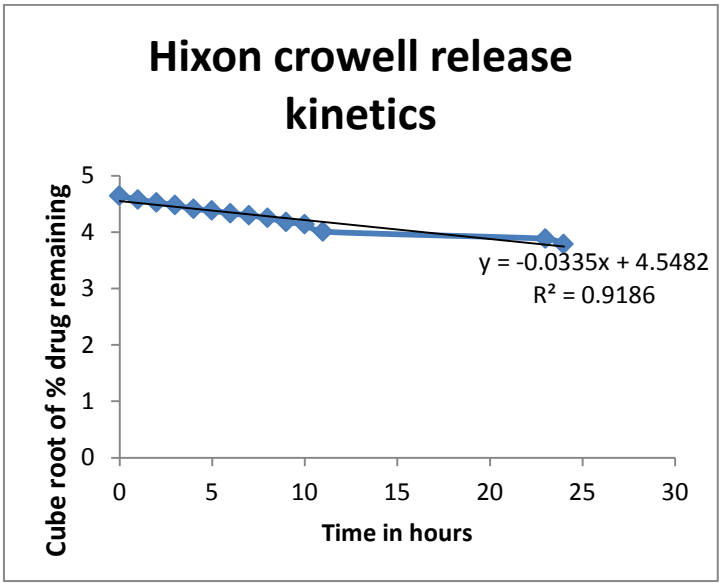


Figure-37:HixonCrowellReleaseKinetics of F7

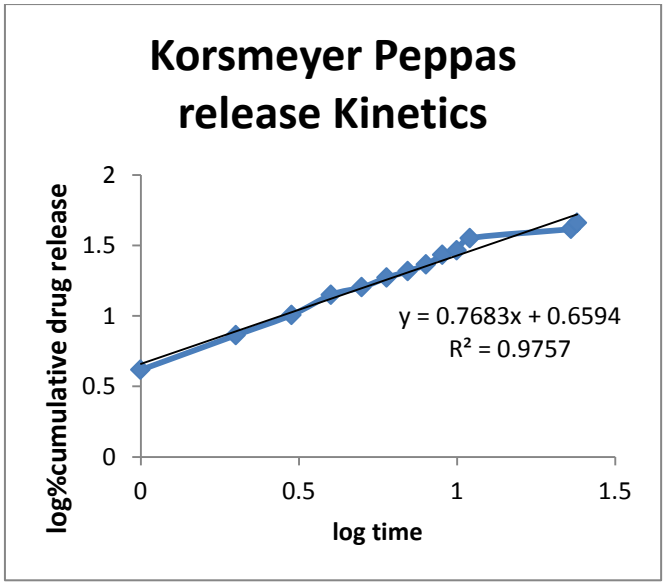


Figure-38:KorsmeyerPeppas ReleaseKinetics of F7

Table-25: Release kinetics of F7

S.No.	Release Kinetics	X-axis	Y-axis	Slope	Intercept	R^2	Linear Equation
1.	Zero order equation	Time in hours	Cumulative % drug release	1.770	6.651	0.892	y = 1.770x + 6.651 R^2 = 0.892
2.	First order equation	Time in hours	Log% cumulative drug release	-0.010	1.975	**0.929**	y = -0.010x + 1.975 R^2 = 0.929
3.	Higuchi release kinetic	Square root of time	Cumulative % drug release	10.09	-4.640	0.958	y = 10.09x - 4.640 R^2 = 0.958
4.	Hixon Crowell equation	Time in hours	Cube root of % drug release	-0.033	4.548	0.918	y = -0.033x + 4.548 R^2 = 0.918
5	Korsmeyer Peppas equation	Log time	Log % cumulative drug release	0.768	0.659	**0.975**	y = 0.768x + 0.659 R^2 = 0.975

Inference

- Formulation F7 followed first- kinetics and their R^2 value 0.929 indicating the release to be dose dependent.
- The drug release was proportional to the square root of time indicating that Nystatin release from Nanosponge was diffusion controlled.

- The n value for the korsmeyer- peppas for formulation F7 was found to be 0.768. So the formulation F7 non-Fickian type mechanism.
- The Drug release pattern from Formulation F7 follows First order release, Higuchi model and Non-fickian transport.

Table-26 : Release kinetics of Nystatin Nanosponge Gel- G1

Time in Hours	%cum drug release	% drug remaining	Log% cum drug remaining	Square root of time	Log time	Cube root of % drug remaining	Log %cum drug release
0	0	100	2	0	∞	4.641589	∞
1	2.73	97.27	1.987979	1	0	4.59896	0.436163
2	5.18	94.82	1.9769	1.414214	0.30103	4.560018	0.71433
3	7.39	92.61	1.966658	1.732051	0.477121	4.524312	0.868644
4	8.62	91.38	1.960851	2	0.60206	4.504193	0.935507
5	9.28	90.72	1.957703	2.236068	0.69897	4.493323	0.967548
6	11.03	88.97	1.949244	2.44949	0.778151	4.464243	1.042576
7	12.66	87.34	1.941213	2.645751	0.845098	4.436812	1.102434
8	14.24	85.76	1.933285	2.828427	0.90309	4.409895	1.15351
9	15.05	84.95	1.929163	3	0.954243	4.395967	1.177536
10	15.81	84.19	1.925261	3.162278	1	4.382818	1.198932
11	16.59	83.41	1.921218	3.316625	1.041393	4.369241	1.219846
23	21.75	78.25	1.893484	4.795832	1.361728	4.277218	1.337459
24	23.15	76.85	1.885644	4.898979	1.380211	4.251556	1.364551

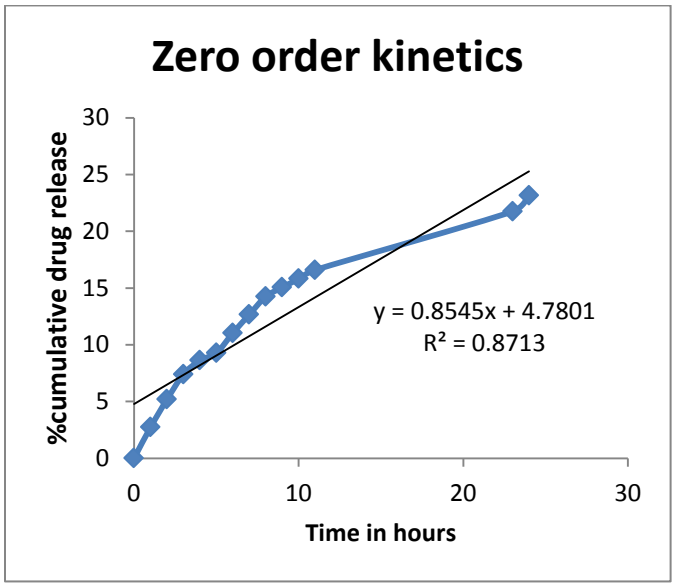


Figure-39: **Zero OrderKineticsof G1**

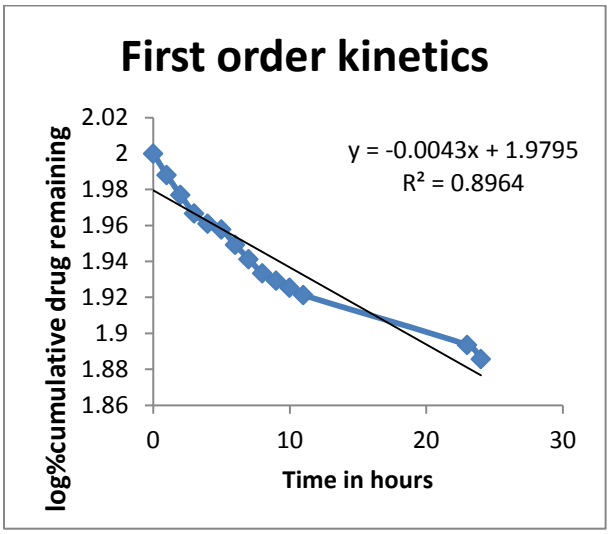


Figure-40: **First orderKineticsof G1**

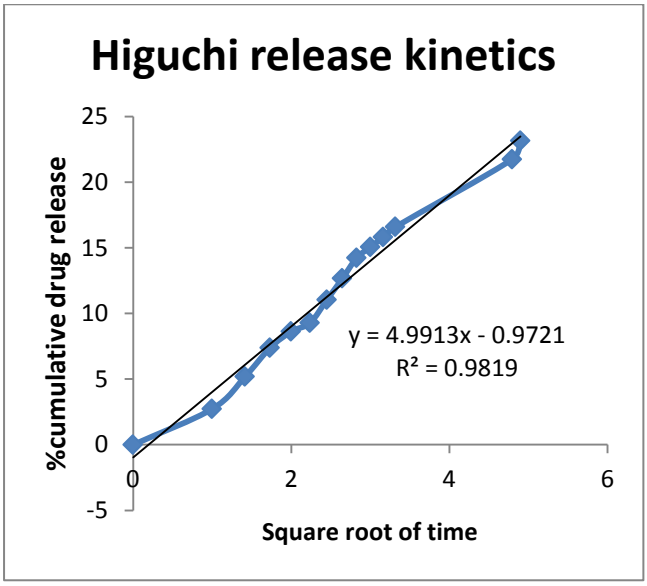


Figure-41: Higuchi Release kinetics of G1

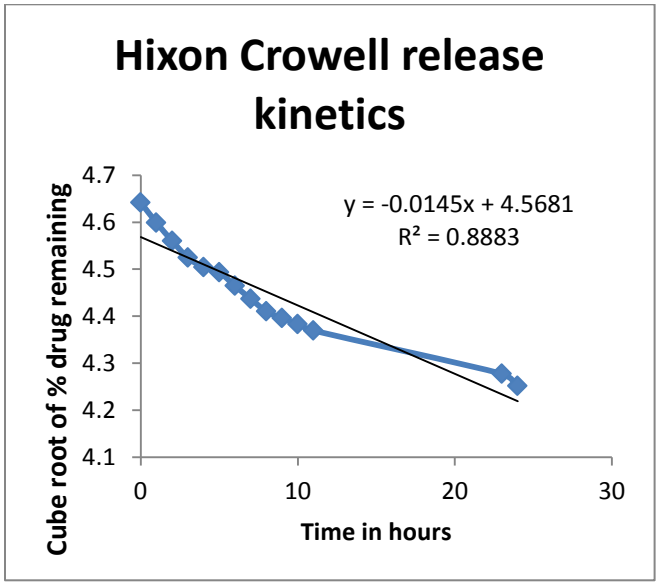


Figure-42: Hixon Crowell Release kinetics of G1

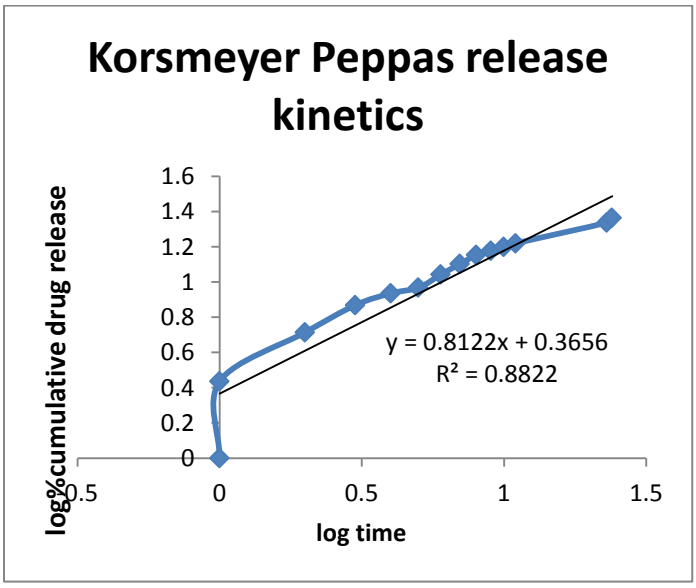


Figure-43 :KorsmeyerPeppas release kinetics oh G1.

Table-27 : Release kinetics of G1

S.No.	Release Kinetics	X-axis	Y-axis	Slope	Intercept	R^2	Linear Equation
1.	Zero order equation	Time in hours	Cumulative % drug release	0.854	4.780	0.871	y =0.854x+4.780 R^2 = 0.871
2.	First order equation	Time in hours	Log% cumulative drug release	-0.004	1.979	**0.896**	y = -0.004x + 1.979 R^2 = 0.896
3.	Higuchi release kinetic	Square root of time	Cumulative % drug release	4.991	-0.972	**0.981**	y =4.991x -0.972 R^2 = 0.981
4.	Hixon Crowell equation	Time in hours	Cube root of % drug release	-0.014	4.568	0.888	y = -0.014x + 4.568 R^2 = 0.888
5	Korsmeyer Peppas equation	Log time	Log % cumulative drug release	0.812	0.365	0.882	y =0.812x+0.365 R^2 = 0.882

Inference

- Formulation G1 followed first- kinetics and their R^2 value 0.896 indicating the release to be dose dependent.
- The drug release was proportional to the square root of time indicating that Nystatin release from Nanosponge gel was diffusion controlled.
- The n value for the korsmeyer- peppas for formulation G1 was found to be 0.812. So the formulation G1 non-Fickian type mechanism.

- The Drug release pattern from Formulation G1 follows First order release, Higuchi model and Non-fickian transport.

Table-28 : Release kinetics of Nystatin Nanosponge Gel-G2

Time in Hours	%cum drug release	% drug remaining	Log% cum drug remaining	Square root of time	Log time	Cube root of % drug remaining	Log %cum drug release
0	0	100	2	0	∞	4.641588	∞
1	3.18	96.82	1.985965	1	0	4.591857	0.502427
2	5.36	94.64	1.976075	1.414214	0.30103	4.557131	0.729165
3	7.28	92.72	1.967173	1.732051	0.477121	4.526103	0.862131
4	9.73	90.27	1.955543	2	0.60206	4.485881	0.988113
5	10.73	89.27	1.950706	2.236068	0.69897	4.469255	1.0306
6	11.37	88.63	1.947581	2.44949	0.778151	4.458549	1.05576
7	14.3	85.7	1.932981	2.645751	0.845098	4.408866	1.155336
8	16.06	83.94	1.923969	2.828427	0.90309	4.378476	1.205746
9	17.28	82.72	1.917611	3	0.954243	4.35716	1.237544
10	18.17	81.83	1.912913	3.162278	1	4.341477	1.259355
11	19.1	80.9	1.907949	3.316625	1.041393	4.324967	1.281033
23	26.04	73.96	1.868997	4.795832	1.361728	4.197579	1.415641
24	28.88	71.12	1.851992	4.898979	1.380211	4.143149	1.460597

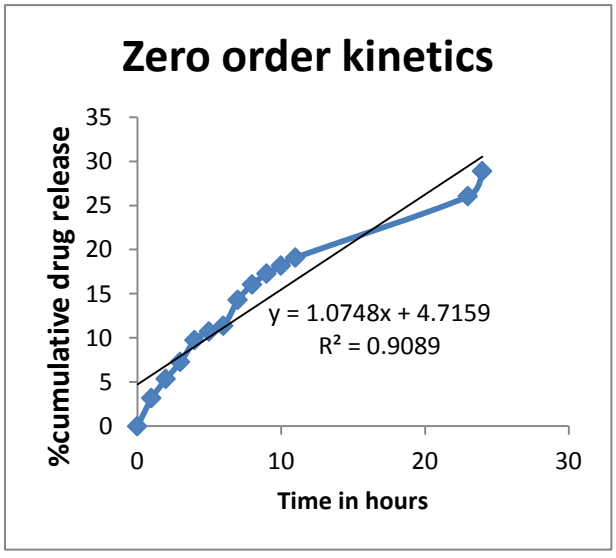


Figure-44: Zero order kinetics of G2

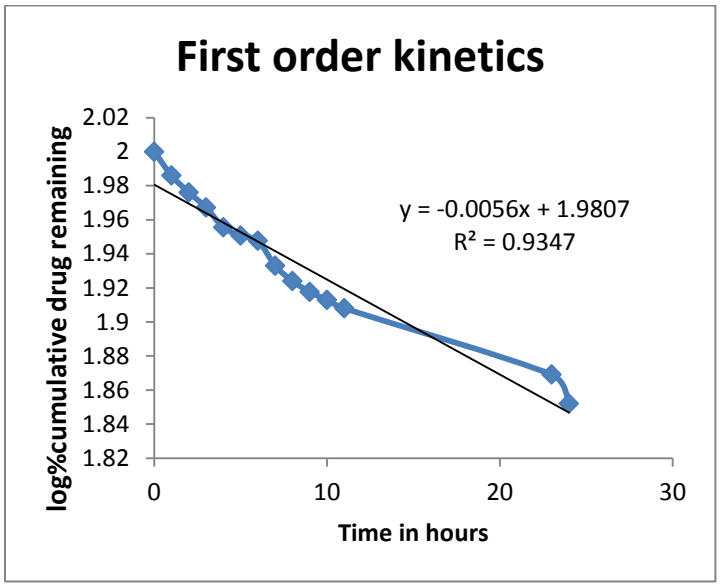


Figure-45 :First order kinetics of G2

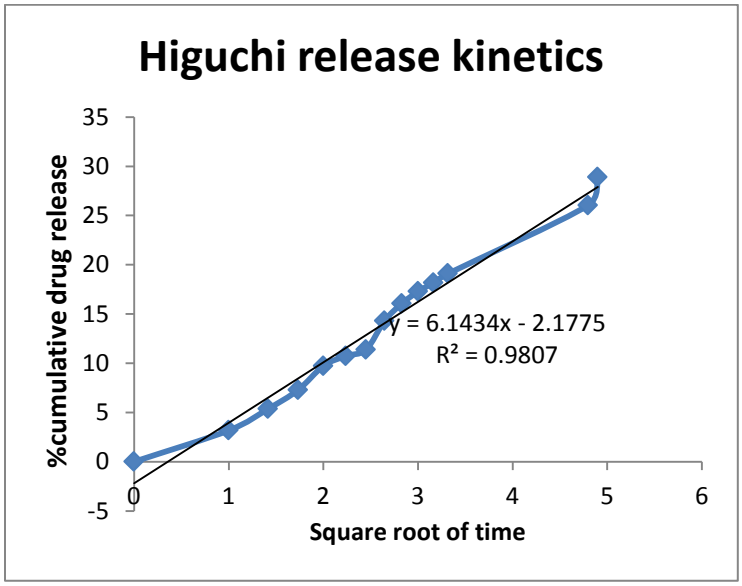


Figure-46: Higuchi release kinetics of G2

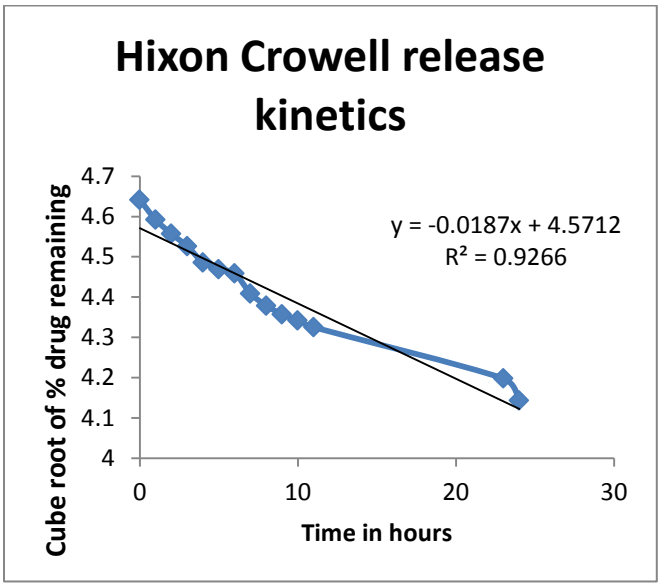


Figure-47 :Hixon Crowell release kinetics of G2

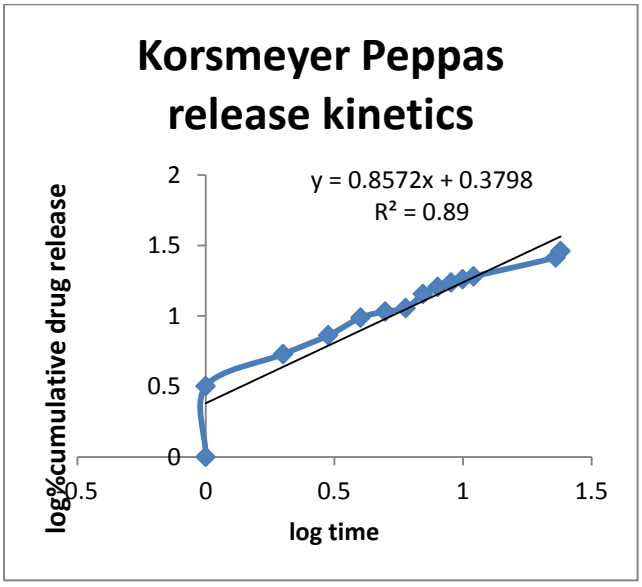


Figure-48:KorsmeyerPeppas release kinetics of G2

Table-29 : Release kinetics of G2

S.No.	Release Kinetics	X-axis	Y-axis	Slope	Intercept	R^2	Linear Equation
1.	Zero order equation	Time in hours	Cumulative % drug release	1.074	4.715	0.908	y =1.074x+4.715 R^2 = 0.908
2.	First order equation	Time in hours	Log% cumulative drug release	-0.005	1.980	**0.934**	y = -0.005x +1.980 R^2 = 0.934
3.	Higuchi release kinetic	Square root of time	Cumulative % drug release	6.143	-2.177	**0.980**	y =6.143x -2.177 R^2 = 0.980
4.	Hixon Crowell equation	Time in hours	Cube root of % drug release	-0.018	4.571	0.926	y = -0.018x + 4.571 R^2 = 0.926
5	KorsmeyerPeppas equation	Log time	Log % cumulative drug release	0.857	0.379	**0.89**	y =0.857x+0.379 R^2 = 0.89

Inference

- Formulation G2followed first- kinetics and their R^2 value 0.934 indicating the release to be dose dependent.
- The drug release was proportional to the square root of time indicating that Nystatin release from Nanosponge gel was diffusion controlled.
- The n value for the korsmeyer- peppas for formulation G2 was found to be 0.857. So the formulation G2 non-Fickian type mechanism.

- The Drug release pattern from Formulation G2 follows First order release, Higuchi model and Non-fickian transport.

Table-30: Drug Release Kinetics for optimized Formulation and their Gel

The regression coefficient (R^2)was determined using the drug release data. The results were shown in the Table :

FORMULATION CODE	ZERO ORDER	FIRST ORDER	HIGUCHI MODEL	HIXSON CROWELL	KORSMEYER PEPPAS	
	R^2	R^2	R^2	R^2	R^2	n
F3	0.914	0.944	0.973	0.935	0.975	0.793
F7	0.892	0.929	0.958	0.918	0.975	0.768
G1	0.871	0.896	0.981	0.888	0.882	0.812
G2	0.908	0.934	0.980	0.926	0.89	0.857

Inference

- **A**ll the formulations followed first- order kinetics and their R^2 value lied between 0.896 to 0.944 indicating the release to be dose dependent.
- The drug release was proportional to the square root of time indicating that Nystatin release from Nanosponge and Gel was diffusion controlled.
- The n value for the korsmeyer- peppas for formulation F3, F7,G1 and G2 was found to be lied between 0.768 to 0.857.. So the formulation F3, F7,G1 and G2 follows non-Fickian type mechanism.
- The Drug release pattern from Nystatin loaded Nanosponge follows First order release, Higuchi model and Non-fickian transport.
- The release of Nystatin Nanosponge gel occurs through the First order, Higuchi model and non – fickian diffusion mechanism.

ANTI FUNGAL ACTIVITY

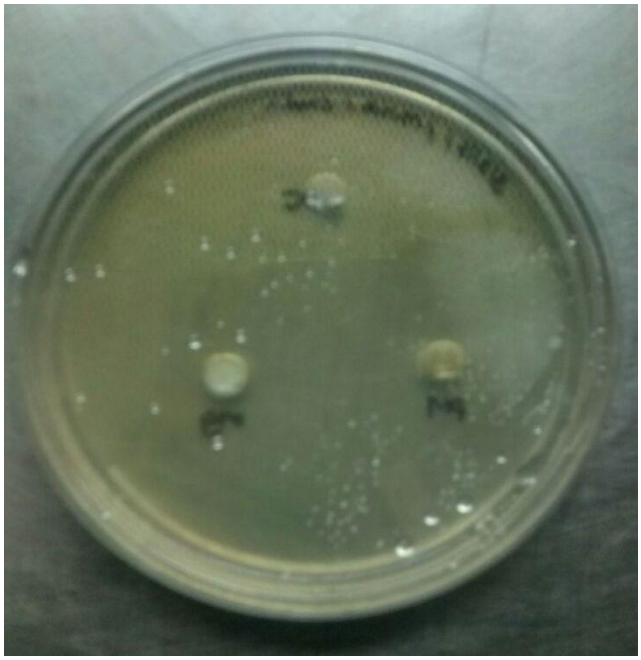


Figure-49 : After inoculation,0 min growth.

Figure-50: After 48 hrs of inoculation of G1

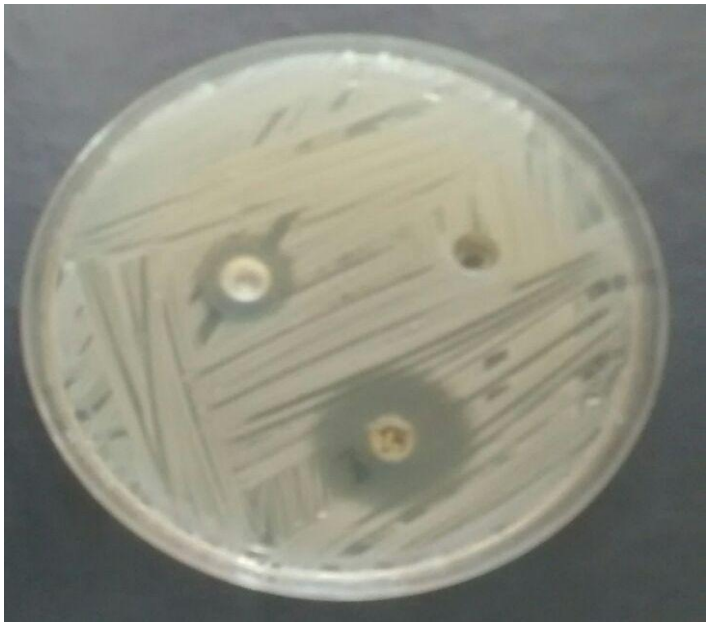


Figure-51:After 48hrs of inoculation for G2

Figure-52: After 48 hrs of inoculation for Marketed sample (Nystatin cream), G1 and G2

Evaluation of Gel

Table-31: Evaluation of Gel

S.No.	Code	Physical appearance	%Drug content	Viscosity (cps)	pH	%Drug release
1.	G1	Good	91.10	2.939×10^6	4.89	25.15
2.	G2	Good	94.55	2.853×10^6	4.92	28.88

Antimicrobial activity of NystatinNanosponge Gel

Table-32: Antimicrobial activity of NystatinNanosponge Gel

S.No.	Formulation code	Anti fungal activity(Zone of inhibition) in mm
1.	Control	0
2.	G1	18±0.8164
3.	G2	19.33±1.2472
4.	Marketed sample	11.667±0.4714

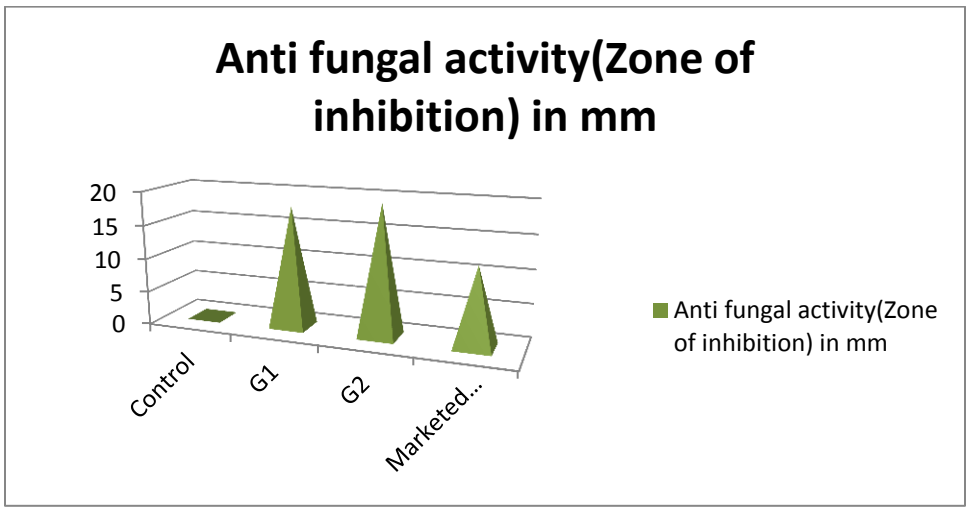


Figure-53: Antifungal activity.

Inference

- The antifungal activity for Nystatin Nanosponge Gel G2 Zone of Inhibition was 19.33mm, for G1 Zone of Inhibition was 18mm and the zone of inhibition obtained for Marketed sample was 11.667mm. The antifungal activity of the Nanosponge gel showed enhancement of Nystatin antifungal effect.

- The purpose of this research was to prepare Nystatin loaded Nanosponge gel for Sustained release of drug, increase the drug solubility, increase the drug permeability, to reduce the dosing frequency and side effects.

- The FTIR studies proved that there were no interaction between the drug and Polymers.

- Homogenization technique followed by centrifugation was employed to prepare Nanosponge using various polymers.

- The formulation were prepared using different Polymers (Ethylcellulose and Polymethyl methacrylate) in different ratios (Drug:Polymer–1:1,1:2,1:3,1:4 and1:5,) Using dichloromethane as cross linker as well as solvent.

- The formulations were characterized for drug entrapment efficiency. The entrapment efficiency of the formulations was observed to be between 97.85 to 99.21. The highest entrapment efficiency was observed with 99.21 and 98.94 for the formulations F3 and F7.

- The formulations were characterized for drug content. The Drug content of the formulations was observed to be between 82.90 to 95.71.

- The particle size analysis done by Malvern Zeta sizer showed that the average particle size of Nystatin loaded Nanosponge F3 and F7 was 231.1 nm and 370.3 nm respectively.

- The SEM analysis of Nanosponge shows the spherical surface of the particles.

- The *in-vitro* release of Nystatin Nanopsonge optimized formulation F3wasfound tobe 36.28% andF7 was 45.66 % at theend of 24 hours.
- The drug content of the Gel G1 and G2 was found to be 25.15% and 28.88 % respectively.
- The *in-vitro* release of Nystatin Nanosponge Gel formulation G1wasfound tobe 23.15% andG2 was 28.88 % at theend of 24 hours.
- The pH of the gels G1and G2 was found to be 4.89 and 4.92 respectively.
- The Viscosity of the gels G1 and G2 was found to be 2.939x 10^6 cps and 2.853x 10^6 cps respectively.
- Anti-fungal activity of Nystatin Nanosponge Gel G2 showed highest zone of inhibition followed by gel G1 and Marketed Sample as **Marzouk M . A.** ***et al*** describes the increased zone of inhibition [45].
- It was concluded that the Nystatin loaded Nanosponge Gel may have increased the solubility, drug release and Antifungal activity(Increase in Zone of Inhibition), and provide Sustained effect.

FUTURE SCOPE

- To perform *in-vivo* study.
- To perform *ex-vivo* study.
- To determine the dosage regimen of the formulation.
- To do effect of Anti fungal activity to other species.
- To develop the Oral dosage form.
- To carry out the IVIVC.
- To perform Toxicity studies.

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